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EXPERIENCE WITH THE ACTINOMYCETACEAE (NOCARDIA)*

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It was from the post-mortem table that we were afforded the opportunity to study a member of this group of micro-organisms. In doing so, we found, as is usually the case that we were able to learn much concerning the isolation and identification of the various other members of the Order in which we were working. It is with this in mind that we wish to pass on our experience to this group—knowing that the responsibility for the isolation and identification of these organisms lies heaviest here.

While more attention has been given to mycological diagnosis in recent years, there is still a need for, "brave hearts" in the clinical laboratory who will attempt the same. It is an added burden perhaps to the busy laboratory but a necessary one and at the same time a very rewarding one. Dr. Haley in one of her reports, tells us of her knowing several competent bacteriologists who nonchalantly discarded all plates on which molds were growing.[1]

It is a fact that any small hospital which is set up for bacteriology can do mycology as well. To have on hand a few special media and the willingness of a worker to learn are the only requisites.

The sad fact is that most of our diagnoses made within this group of organisms comes too late to be of much benefit to the doctor or the patient. The prognosis of the patient depends on the speedy and accurate identification of these organisms. Antibiotic and/or chemotherapy are effective before the infection becomes generalized.[2]

Table I shows the Order under discussion. You will note that the family Mycobacteriaceae is included in this Order. We know that the genus Mycobacterium is made up of acid-fast organisms whose morphology may be in certain instances somewhat variant. Within the family Actinomycetaceae we have the genus Nocardia which often has species or strains which are also acid-fast. Many cases of nocardiosis have been diagnosed as tuberculosis.[3] The treatment is certainly different in each case. We should be convinced that in every case where

* 3rd ASMT Award, 1957. Read before 25th ASMT Convention, Chicago, Illinois, June, 1957.

tuberculosis is suspected, specimens should be set up for mycology as well. These are all granulomatous diseases which do not show up differentially by X-ray, PAS and ordinary H & E tissue sections.[3]

TABLE I

ORDER:	ACTINOMYCETALES*
Family I	MYCOBACTERIACEAE
	Genus Mycobacterium
Family II	ACTINOMYCETACEAE
	Genus Nocardia
	Actinomyces
Family III	STREPTOMYCETACEAE
	Genus Streptomyces

*Bergey's Manual

The isolation and identification of this organism began with direct smears from the brain abscess obtained at autopsy. The Gram stained smear showed large numbers of polymorphonuclear cells with an occasional clump of entangled, hair-like filaments staining Gram positive. Some areas showed clumps with a heavily stained central mass, while others were looser with only the filaments weaving in and out among the cells. The filaments were beaded terminally and intact—suggestive of the streptococcal chain but appearing to be more consistently a part of the whole filament. No bacterial forms were noted. These characteristics suggested the old term "Streptothrix".

Some of the exudate had been collected in broth. This showed a number of small, greyish-white granules visible macroscopically. Microscopically these granules appeared to be identical morphologically with the clumps seen on the direct smear.

Material from the broth was inoculated to blood agar plates. One was incubated aerobically and the other anaerobically. Within 48 hours small dull, greyish, cerebriform colonies were discernible on both plates. When smears were made from the colonies, their consistency was noted to be hard. A whole colony was easily removed intact. Gram stained smears of the colony broken up in saline revealed the now familiar masses of Gram positive, hair-like entanglements most of which had the beaded appearance. The organism described from the broth and the direct swear were then assumed to be the same as the one now isolated in pure culture. Acid-fast staining by the Kinyoun method showed the organism to be partially acid-fast.

Up to this point enough information was obtained to be certain that this was not an organism which could be classified among the, "true bacteria". There was, however, enough information to classify it in the Order: Actinomycetales. Of the families in this Order, Mycobacteriaceae was easily eliminated due to the rapid growth and well developed mycelium (microscopically) of our organism. The family Actinomycetaceae might well hold the answer to the problem. The genus Nocardia more specifically tempted committal. Unlike the genus Actinomyces, Nocardia is frankly aerobic and often acid-fast or partially so. It would have been easy to classify the organism in the genus Nocardia except for two points. First, the colonial morphology of this organism was not typical.

Instead of the heaped or folded growth, we had a hard cerebriform colony which developed a powdery dome in 3 or 4 days on any of the common semi-solid media including Sabaroud's dextrose agar. On Sabaroud's the under surface was yellow-orange and the surface was pinkish-white. The second point of possible exception was a very musty odor of freshly turned soil which is more characteristic of the Streptomyces.

We had now eliminated the Mycobacteriaceae and the Actinomyces and were left with the genus Nocardia and the family Streptomycetaceae. Of the Nocardia group, two species were particularly considered. *Nocardia asteroides* var. *gypsoides* was considered because of the powdered dome. *Nocardia brasiliensis* has the powdery surface plus a charactertistic earthy odor and liquifacation of gelatin. Table II summarizes the studies which helped to differentiate this organism from the Streptomycetaceae. Chief among these of diagnostic value was the acid-fastness of this organism. Litmus milk was not coagulated in 60 days while most Streptomyces produce a pigmented ring or pellicle in this media. Microscopic examination of the colonies did not show the sporulation characteristic

TABLE II

Some Differential Characteristics[1]

Mycobacteriaceae
1. Rudimentary or no mycelium
2. No spores
3. Are acid-fast

Actinomycetaceae

Nocardia
1. Aerobic*
2. Acid-fast or partially*
3. Filaments form transverse walls-coccoid cells-microscopic mycelium
4. Colonies usually pasty or mealy
5. Typically no aerial mycelium*
6. Some pigmenters
7. Non-fragmenting mycelium*
8. Litmus milk variable
9. Gelatin—usually no liq. or limited

Actinomyces
1. True mycelium produced*
2. Fragments easily*
3. Anaerobic or microaerophilic*
4. Not acid-fast*
5. Scanty aerial mycelium or none
6. Litmus milk-acid with no coag. or pep.
7. Gelatin—no liq.

Streptomycetaceae
1. Vegetative mycelium, non-fragmenting
2. Not acid-fast*
3. Conidia produced in aerial hyphae in chains*
4. Typical sporulating hyphae:
   a) straight
   b) clustered
   c) whorls or tufts
   d) spirals (open or compact)*
5. Some pigmenters
6. Aerobic
7. Litmus milk—pigment ring or pellicle usually
8. Gelatin—more rapid liq. if.

* Characteristics most significant.

of the Streptomyces. In gelatin, flaky, yellowish colonies grew well in the upper-most portion with no liquifaction at 60 days. A very loose pellicle was formed in peptone broth with very slight darkening of the media in 14 days.

Carbohydrate studies were as follows: sacchrose, maltose and dextrose were fermented—lactose was not fermented. Urea broth was negative. Reference is made to McClung's work here.[6]

The colonial morphology differed in that Streptomyces have a true vegetative mycelium. This organism produced a mycelium clearly visible microscopically only. A guinea pig was inoculated early in the study. A suspension of the organism was inoculated intraperitoneally. The animal died in one week. (Death was attributed to toxicity rather than true invasion of the organism.)

One who isolates these organisms will feel the inadequacy of the present taxonomy. Each small bit of knowledge concerning the growth and biochemical reactions of the unknown organism assumes great importance. As the data accumulates careful evaluation of the facts must be made before the final identification can be made. "A preliminary report of genus Nocardia" can of course be made long before a specie identification is possible. Several good texts and references are readily available for the detail work. It is also a good idea to consult with another worker whenever possible.

Sensitivity studies by the plated-disk method on three different occasions gave fairly consistent results. Table III summarizes these results. A slightly sensitive result is considered to be resistant in this laboratory.

TABLE III
Sensitivity Studies*

Aureomycin	SS	Novobiocin	VS
Chloromycetin	R	Oleandomycin	R
Dihydrostreptomycin	R	Penicillin	R
Erythromycin	SS	Sulfa Drugs	S
Furadantin	SS	Terramycin	R
Neomycin	R	Tetracycline	SS

* R-resistant; S-sensitive; SS-slightly sensitive; VS-very sensitive.

POST MORTEM

Pertinent laboratory findings were as follows: WBC 14,900, Spinal fluid cell count 1,590 neutrophils, Spinal fluid protein 148 mg/%, Spinal fluid and Blood cultures gave no growth.

Post mortem examination revealed a brain abscess of the occipital lobe with extension and involvement of the right choroid plexus and pneumonia of the left upper lobe. Microscopic examination of the brain revealed abscess formations and many multinucleated giant cells. Sections of the lung showed pneumonitis with a few multinucleated giant cells. No fungi were noted in the H. & E. stained sections.

Conclusion

On the basis of these studies and by the process of elimination of all of these groups in this Order the organism was identified as a Nocardia asteroides var. gypsoides. Two other independent workers arrived at the same conclusion.[7,8]

This patient came to us one month after an episode of "pneumonia". Sputum from the patient at that time might have produced the same organism which we recovered at autopsy. At that time the patient might have had a good chance for recovery.

Summary

The direct smear prepared from a brain abscess at autopsy revealed a "Streptothrix" type of organism. From the exudate the organism was isolated and identified as a member of the Order: Actinomycetales. A summary of the histopathology is given. The identification of the organism as a Nocardia asteroides var. gypsoides was arrived at after the elimination of the various members of this Order. The process of elimination is given in some detail as experience for the isolation and identification of these organisms. Sensitivity studies are included.

Acknowledgment

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THE ROLE OF THE LABORATORY IN THE DIAGNOSIS OF FIBROCYSTIC DISEASE OF THE PANCREAS*

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Fibrocystic disease of the pancreas is an hereditary disease of children in which pancreatic insufficiency is only one feature. It is now believed that many and perhaps all exocrine glands or glands of external secretion are affected. Involvement of the lungs, liver, sweat and salivary glands as well as the pancreas gives rise to manifold clinical manifestations. Laboratory examinations of various body secretions and excretions are extremely instrumental in establishing the correct clinical diagnosis.

At the present time there are several names used to describe this progressive disease. Fibrocystic disease of the pancreas, cystic fibrosis of the pancreas and pancreatic fibrosis all connote that the disease involves the pancreas alone. The name mucoviscidosis implies a condition in which a basic defect resides in the mucus secreting glands, whereby the fluid formed has an increased viscosity. Authorities have been unable to decide on a conclusively descriptive term. This generalized disorder in young children accounts for virtually all cases of pancreatic deficiency, and for a majority of those with chronic (non-tuberculous) lung disease.

The hereditary nature of the disease has been studied. It is thought that it is determined by a recessive gene consequently there may be more than one child in a family affected. Most patients do not survive to age 10.

The confirmation of the diagnosis of fibrocystic disease of the pancreas is established as a result of laboratory investigation. The seriousness of the disease, the emotional impact on parents, and heavy expenses make it extremely important that no errors be made. Many times if an early diagnosis can be made, proper treatment with pancreatin for the pancreatic defect, and with antibiotics to combat the pulmonary infection may prolong the life of the patient. The following diagnostic procedures are being used.

Gross and microscopic examination of stools may be a primary simple test in establishing a diagnosis of fibrocystic disease. Because of the absence of pancreatic enzymes, foodstuffs are poorly digested and absorbed. Fats are not split and large amounts of neutral fat are found in the stool. The stools are bulky, greasy and extremely foul. The child will be in a state of malnurition in spite of apparently adequate diet.

Until very recently when diSant'Agnese[1,2,3] proved that the sweat electrolytes were abnormally high in the disease, the definitive clinical diagnosis depended on studies done upon the duodenal fluid. With pancreatic deficiency there is marked decrease to a complete absence of pancreatic enzymes. The clinician has been most interested in trypsin in the past because it was felt that its measurement was most reliable and reflected the activity of the other digestive enzymes.

Because duodenal intubation is a tedious and sometimes difficult task, which must be done by an experienced operator, many workers have

* Claudia M. Stannard Award, 1957. Read before 25th ASMT Convention, Chicago, Illinois, June 1957.

attempted to eliminate the test as a diagnostic tool. The simplest procedure, used as a screening test, is the examination for *stool trypsin*. Normal infants have a significant amount of tryptic activity in the feces. A 1:100 dilution is capable of digesting the gelatin surface from X-ray film at 38 degrees C. in one hour.[4] Three negative results of trypsin examinition in an infant in the 1:5 or 1:10 dilution are considered sufficient evidence to justify a tentative diagnosis. A control is always run with each sample. Some patients with proven complete pancreatic insufficiency give a positive stool trypsin test. Johnstone and Neter[5] explain this by the presence of gelatin liquefying bacteria in the feces. They have formulated several tests to differentiate between bacterial and tryptic gelatin liquefaction in the feces.[6] Prolonged use of antibiotics for treating the respiratory infections of the disease may alter the intestinal flora so that proteolytic bacteria may emerge as predominant microorganisms in the stool. *Proteus morganii* and *Pseudomonas aeruginosa* arise as the greatest offenders. The stool trypsin test is never used to make a final diagnosis.

A widely used method of measuring direct *tryptic activity* in the duodenal fluid is the Anderson and Early modification of the Fermi method.[7] Its ease of performance makes it readily adaptable to routine use in the clinical laboratory. Dilutions of duodenal fluid are incubated at 37 degrees C. for one hour with 2 ml. of a 7.5% solution of Knox gelatin substrate in a final volume of 4 ml. At the end of this period the tubes are chilled. The end point is determined by noting the tube in which the gelatin remains liquid. If there is not sufficient trypsin in the duodenal fluid, the gelatin will retain its property of solidifying at 3 degrees C. A control must always accompany each test. An objection to this method is that the end point depends upon the digestion of over 85% of the substrate and moderate reduction in enzymatic activity remains undetected. The amount of gelatin actually digested can be readily determined by observing the change in viscosity of the original solution. The gelatin-viscosity method described by Leubner and Shwachman[8] is sufficiently quantitative to detect partial pancreatic insufficiency.

Research is being conducted to determine the relative amounts of other enzymes such as amylase, lipase and chymotrypsin in the duodenal fluid. It has been noted that in some cases of partial pancreatic insufficiency there is still measurable trypsin while several of the other enzymes are all but lacking.

The duodenal fluid obtained from patients with fibrocystic disease is scanty and viscid. The alteration in viscosity may precede the disappearance of pancreatic enzymes from the fluid; this measurement may be an aid in the diagnosis of partial pancreatic insufficiency. The duodenal fluid collected for trypsin determination may be used for this test. *Viscosity* is determined with an Ostwald viscosimeter that is standardized so that distilled water at 20 degrees C. will have a flow time of 32 or 33 seconds. The flow time of duodenal fluid of normal healthy children is from 1-1½ minutes. In patients with fibrocystic disease, the fluid may be too viscid to measure. It was over three minutes in 87% of tested cases.[9]

The finding of an *abnormal electrolyte concentration* in sweat of patients with fibrocystic disease has furnished a valuable diagnostic tool in the

investigation of the disease. The introduction by Shwachman of a plastic bag to heighten sweating in patients, instead of a constant temperature room, has further simplified the procedure and brings it within reach of most hospital laboratories.

The values for sweat chloride and sodium are markedly increased in this disease. The values are above those associated with other kinds of disease. The same holds true of the potassium content of the sweat although not to the same degree. diSant'Agnese, et al[1,2,3] have found in their studies that the sodium and chloride values in abdominal sweat of patients was 2-4 times as high as in controls.

CHLORIDES		
Patients	60-160 MEQ./L.	(mean 106 MEQ./L.)
Controls	4- 80 MEQ./L.	(mean 32 MEQ./L.)
SODIUM		
Patients	80-190 MEQ./L.	(mean 133 MEQ./L.)
Controls	10-120 MEQ./L.	(mean 59 MEQ./L.)

Abdominal sweat has been found to be more constant in results. Sweat is collected on a previously weighed gauze pad which is taped to the abdomen of the patient enclosed in a plastic bag to heighten sweating. The sweat is extracted from the pad with distilled water and the chlorides determined by the method of Schales. The sodium is determined with the flame photometer.

A common symptom resulting from these increased electrolyte levels in the sweat is a condition of heat exhaustion in hot weather. This physiological disturbance many times will affect the entire body fluid electrolyte balance.

Only recently a new test for detecting the approximate chloride content of the skin surface has been devised by Shwachman and Gahm[10]. The test meduim is composed of silver nitrate and potassium chromate suspended in agar. When little chloride is present on the skin surface of the hand or foot, the imprint on the agar plate is barely detected. When considerable chloride is present, an intense whitish-yellowish discoloration of the imprint occurs immediately. This test was devised to provide an opportunity to screen large numbers of children for fibrocystic disease. The test is simple, painless, takes little time and is inexpensive.

The secretory rate of the parotid glands is markedly increased above normal in this disease. The electrolyte concentration of saliva is also abnormally increased. However, *salivary electrolyte analysis* is not too useful in differential diagnosis. There are many normal high controls and low patients. The saliva analysis is only another tool in establishing a positive diagnosis of fibrocystic disease of the pancreas.

Our laboratory, in cooperation with members of the local Cystic Fibrosis society, conducted a study on 27 children. Seven of these children had previously been diagnosed as cases of fibrocystic disease; the other twenty were from the same families as the affected children. Using methods cited above, tests for sweat sodium, chloride and saliva chloride were run.

All of the siblings had normal electrolyte values while six of the seven

patients had extremely high sodium and chloride concentrations in the sweat and saliva. One child had been diagnosed as a positive fibrocystic on the basis of clinical symptoms and a high salivary chloride level. The results of our tests showed her saliva chloride concentration to be high but her sweat electrolytes to be within normal range. Her physician has since altered his original diagnosis.

Our study confirmed the work done by diSant'Agnese and others. We found the sweat electrolytes to be uniformly high in patients with fibrocystic disease. The salivary chloride test was not found to be a dependable diagnostic tool. Control of extraneous factors is difficult especially in very young children and many times the results do not correlate with the sweat electrolyte findings.

In almost all cases of the disease, pulmonary involvement of a varying degree occurs at some time during the course of the illness. The respiratory symptoms are usually severe and account for a large majority of the deaths.

There is increased mucus production in the bronchi and the inability to remove it leads to widespread bronchial obstruction and secondary bacterial infection follows. The infection may appear clinically as bronchitis, sinusitis, pneumonitis, bronchopneumonia and others.

Throat and nasopharyngeal cultures yield *Micrococcus pyogenes var. aureus* with few exceptions. However, prolonged therapy with wide-spectrum antibiotics may alter the predominating flora. Garrand,[11] et al, found several cases in which *Pseudomonas aeruginosa,* ordinarily of low pathogenicity, became prominent and invasive as a result of prolonged antibacterial therapy. The susceptible gram-positive organisms are eliminated and the resistant proteolytic gram-negative bacteria may take their place. These latter organisms may be responsible for the terminal stages of the disease.

Summary

The laboratory has a very important role to play in the diagnosis of fibrocystic disease of the pancreas. The relatively simple screening tests are usually requested first. The gross and microscopic examination of the feces and the stool trypsin test give some indication of the condidition of the pancreas. The test for direct tryptic activity in the duodenal fluid has been replaced almost entirely by tests for high electrolyte concentrations in sweat. However, it is still a useful tool in making a diagnosis of the disease, especially, in determining the degree of pancreatic involvement. The most valuable diagnostic procedure is analysis of sweat for sodium and chloride levels. The extremely high electrolyte concentration is a unique charactertistic of this disease. Bacteriological studies are carried out to determine the micro-organism(s) causing the pulmonary infection.

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IN MEMORIAM

Betsy C. Adriance, Galveston, Texas	November, 1957
Sister M. Adelgundis Forst, Garrett, Indiana	November, 1957
Emelia Lanzetta, West Palm Beach, Florida	December, 1957
Marjorie Moss Jones, Washington (state)	December, 1957
Agnes Clements	January, 1958
Sister M. Athanasius Elwood, Jacksonville, Illinois	February, 1958
Dr. Edward Mugrage, Denver, Colorado	March, 1958

Death, to a good man, is but passing through a dark entry, out of one little dusky room of his father's house, into another that is fair and large, lightsome and glorious, and divinely entertaining.

—Clarke

PRACTICAL IMMUNOHEMATOLOGY PROCEDURES FOR THE ROUTINE LABORATORY*

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Immunohematology procedures when correlated with the findings of the routine hematology study may often provide valuable information in cases where a hemolytic process is suspected. In hemolytic anemia we discriminate between those cases due (1) to intrinsic defects of the red cells and (2) to extrinsic factors which attack the normal red cell. The first group consists of congenital abnormalities such as hereditary spherocytosis, Mediterranean anemia, and sickle-cell anemia. In these disorders the red cell produced by the marrow is inherently or intrinsically defective and as such is vulnerable to premature destruction. The techniques described in this paper are usually negative in cases of intrinsic defects. However, there are instances where an acquired hemolytic process develops in addition to the primary disease and positive results have been reported.

Extrinsic mechanisms resulting in increased cell destruction are numerous and varied. The malarial parasite, Bartonella bacillus, B. Welchii, certain viruses and chemicals can apparently act upon the red cells directly, leading to their destruction. Of great interest, however, is the immunologic mode of attack, seen clinically in the form of both iso-immune and auto-immune disorders. Iso-immune hemolytic disease is typified by erythroblastosis fetalis, in which an antibody is built up in the mother which is injurious to the red blood cells of her own infant. In the auto-immune disorders (acquired hemolytic anemia), antibodies are built up which are injurious to the individual's own cells. The methods of auto-immunization are quite obscure but it is postulated by some that certain red cells may become sufficiently altered to become antigenic. Alterations in red cell surface may conceivably occur when the cells are exposed to certain viral or other infections, or to chemicals or even malignant neoplasms or leukemias.[1] Under such circumstances a specific protein (antibody) is built up against these new antigens.

Another mechanism might be through that of a hapten (virus, chemicals, etc.) combining with the cell, the hapten cell combination thus becoming antigenic and producing a specific abnormal protein. Such a situation is seen in the development of the quinidine platelet antibody in indviduals developing thrombocytopenic purpura upon repeated administration of quinidine.[2]

Dacie[3] believes that during the course of disease the reticuloendothelial system may produce abnormal proteins which behave like erythrocyte antibodies. The primary abnormality is thus a pathological reaction from the antibody—forming tissue rather than a change localized in the erythrocytes.[3]

The opinion has become prevalent that once an erythrocyte auto-antibody has been demonstrated in hemolytic anemia, this antibody can be held responsible for the disease of the patient. Similar views are held with regard to the leukocyte and thrombocyte antibodies which have

* 1st SPF Award in Serology, 1957. Read before the 25th Anniversary Convention, ASMT, Chicago, Illinois, June 1957.

been demonstrated with increasing frequency during recent years in leuko—and thrombocytopenia.

The erythrocyte antibody has the capacity to attack not only the individual's own cells but red cells of all humans, whatever the blood group. There have been a few cases reported where specific antibody, i.e., anti-c, anti-e, were identified. These specific auto-antibodies react only with the patient's own blood cells and cells that contain the homologous antigen.[4] These findings would suggest that increased destruction of the erythrocytes begins before the auto-immunization. This erythrocyte destruction, which is not referable to antibodies may liberate more specific erythrocyte antigens than normal. In predisposed individuals with an unusual propensity for developing antibodies this may give rise to more auto-antibody formation, often with specific components. In this hypothesis only secondary importance is thus ascribed to the auto-antibody.

The auto-antibody appears to be firmly attached to receptor groups on the red blood cell and in vitro cannot be displaced by repeated washings with salt solution. Being a globulin, the antibody adherent to the surface of the red cells reacts with human antiglobulin serum (usually prepared in rabbits) with the result that agglutination takes place in the test tube (positive Coombs test). Cells coated with auto-antibody are either injured directly because of this coating or only after becoming agglutinated in masses. Agglutinated red cells are probably immediately vulnerable, and thus easily lysed by such factors as complement and mechanical trauma.

Numerous types of hemolytic antibodies have been differentiated but in general, they may be divided into the following categories: (1) agglutinating: warm and cold, and (2) hemolysins: a number of different varieties may be further distinguished. For example, some agglutinating antibodies are "complete," i.e., will function against red cells in salt solution; while others are "incomplete" and can be demonstrated in vitro only by use of a protein-rich medium. Some cold hemagglutinins appear to react only at low temperatures, whereas, others have a high thermal amphitude (from 3° C. to 30° C.).

In our hematology department any patient who shows clinical evidence of hemolysis has the following blood study. Direct and indirect Coombs test, titration of warm and cold agglutinins, antibody screening with enzyme treated cells and test for cryoglobulin. These tests can be performed on specially drawn clotted blood or on the oxalated specimens sent to the laboratory for routine blood counts.

Coombs Test

The antiglobulin test is one of the most sensitive for the demonstration of the "incomplete" antibodies, such as are found in hemolytic disease of the newborn, blood transfusion reactions and acquired hemolytic anemias.

The Coombs antibody is not a single antibody but a multiplicity of antibodies. Dacie[5] was the first to consider that an antiglobulin serum neutralized with small amounts of gamma globulin failed to react with Rh-sensitized cells or with cells from patients with acquired hemolytic anemia of the "warm-antibody type." The serum would, however, react with cells sensitized by normal cold antibodies or with cells from pa-

tients with acquired hemolytic anemia of the "cold-antibody type."

It is important to realize that the Coombs reagent does not react with a specific antibody, but rather with any human globulin which is attached to the cells. The volume of saline used in washing the cells should be large in comparison with the volume of packed erythrocytes employed; this is essential to the proper performance of the test since insufficient washing may leave traces of serum proteins which react with the antiglobulin serum, causing a weak or falsely negative reading. When there is doubt as to the adequacy of the washing it is preferable to wash excessively rather than insufficiently, since antibodies do not "wash off" in these circumstances.

Test Tube Procedure For The Direct Test:

(1) Wash 0.5 cc. or less of blood in a 13 x 100 mm. test tube with three changes of isotonic saline.

(2) Make a 2 per cent saline suspension from the packed red cells.

(3) Set up three small (10 x 75 mm.) test tubes. In the first, place two drops of undiluted antihuman globulin serum; in the second, two drops of the antihuman globulin serum diluted 1:4 with 0.85 per cent saline; in the third, two drops of the serum diluted 1:8. Label the tubes and to each add one drop of the 2 per cent cell suspension.

(4) Incubate at 37° C. for 30 minutes.

(5) Shake the tubes gently and check for agglutination with the naked eye. If no clumping is visible, check with a hand lens or with the low power of the microscope.

(6) If negative, centrifuge for two minutes at 1000 r.p.m. Check again for agglutination as above.

Positive and negative controls are also carried with this test. The negative control is a 2 per cent suspension of O Rh positive red cells. The positive control cells may be made as follows: Incubate equal parts of a 2 per cent suspension of washed normal group O Rh positive red cells and anti-Rho blocking antibody serum for 30 minutes at 37° C. Wash the cells with four changes of isotonic saline and resuspend as a 2 per cent suspension.

Comment: When small amounts of antibody are fixed on red cells, such as results when red cells are exposed to low concentration of antibody, potent anti-human globulin serums may give negative or significantly weaker reactions than may be obtained if the anti-human serum is diluted.[6] This is a manifestation of the so-called prozone phenomenon. The prozone phenomenon may be obviated by increasing the amount of antigen to a more favorable proportion to the amount of antibody present. Many workers prefer the slide test Coombs because they feel that the 40-50 per cent suspension of cells is not as subject to the prozone phenomenon.

When there is the possibility of the presence of "cold" auto-agglutinins, particularly in disease and occasionally in pregnancy, an additional step in the procedure is required to eliminate the possibility of a falsely positive reaction. After the red cells have been washed and packed, incubate them in a water bath at 37° C. for one to three minutes and add the antiglobulin serum to the cell suspension while the latter is warm. The purpose of this step is to elute the "cold" antibodies from the surface of the red cells.[7]

In the "direct test" unknown red cells are tested to determine if they have been sensitized in vivo; in the "indirect test" a serum under test for the presence of antibodies is allowed to react with specific red cells (usually at 37° C. for one hour) to see if the red cells become sensitized in the process. Another important use of the "indirect test" is to use a known reagent serum against red cells under test to determine the presence of certain agglutinogens not detectable by ordinary agglutination methods, i.e., D^u antigen.

Indirect Coombs Test Procedure—for testing a serum containing unknown antibodies:

(1) Make 2 per cent suspension of red cells of known antigenic content.

(2) Incubate equal quantities of these suspensions and serum at 37° C. for 30-60 minutes.

(3) Wash cells with three changes of isotonic saline and proceed as with the "direct" test.

For testing unknown cell antigens proceed with sensitization as above using known anti-serums.

Comment: Frequently the patient's serum is tested against cells suspended in serum, plasma, or bovine albumin; this is sometimes unsatisfactory since so many patients in need of transfusion have rapid sedimentation rates; the rouleaux formation is difficult to distinguish from true agglutination, and may even mask it. To be certain of the validity of clumping, rouleaux formation must be eliminated entirely; the antiglobulin test does this, since erythrocytes are washed in saline, and the antiglobulin serum is too dilute to support rouleaux.

Titration of Serum Antibodies

These titrations are done to determine the amount of antibody free in the serum in cases where there is a positive "direct" or "indirect" Coombs test.

1. A rack of test tubes 10 x 75 mm. are set up and numbered according to the dilution of the serum in the tubes, as 1:1, 1:2, 1:4, 1:8, etc.

A series of tubes are used for the detection of both the "saline" or complete antibody and the "incomplete" antibody. The saline antibody will react in both saline and albumin. The "incomplete" antibody will cause clumping in albumin, serum, or plasma, but not in saline.

2. Using a 1 cc. pipette deliver to the bottom of all tubes but the first 0.1 cc. of 20 per cent albumin or 0.1 cc. saline.

3. Deliver 0.1 cc. of patient's serum to the first tube and 0.1 cc. to the second tube. Mix the contents of the second tube and discard pipette. With a clean pipette transfer 0.1 cc. from the second tube to the third tube and mix, etc. When the last tube has been mixed, draw up 0.1 cc. and discard.

4. With 1 cc. pipette add 0.1 cc. of a 2 per cent cell suspension to each of the tubes. The type of red cells used is determined by the type of antibodies suspected to be in the serum. If it is a case of specific iso-antibodies then the cells must contain the corresponding agglutinogens. If it is an auto-sensitization then the patient's own cells, cells of the same type as the patient and Type O cells might be used. Cells for the saline titration are washed and suspended in saline. Cells for the protein are suspended in albumin or AB serum.

5. Shake tubes well and place in 37° C. water bath for two hours.
6. After incubation period, shake tubes just enough to dislodge button from bottom of tube. Observe for agglutination macroscopically. Record as end point the tube containing the greatest dilution of serum which causes definite macroscopic agglutination. Check end points and weak agglutination with the microscope.

Comment: The diluent used is of greatest importance in the demonstration of Rh antibodies and other iso-antibodies. The enhancing influence of normal serum as a diluent in the demonstration of immune anti-A, and its relative superiority to serum-albumin mixture is fairly characteristic for immune anti-A and anti-B. Baby's cells which were sensitized by immune anti-A antibodies are better agglutinated in normal adult serum than in the serum-albumin mixture, in direct contrast to cells sensitized by the Rh antibody.[10] In acquired hemolytic anemia the patient's cells agglutinate in either adult serum or serum-albumin. However, the majority of cases react best in the serum-albumin mixture similar to cases of Rh sensitization.

The titration procedures are summarized in Table I.

TABLE I
Titration of "Warm" Agglutinins

Incubate at 37° C.	DILUENT FOR SERUM AND CELL SUSPENSION		
	SALINE		20 percent Albumin or AB Serum
0.1 cc. Dilution of Patient's Serum	0.1 cc. Washed Cells	0.1 cc. Enzyme Treated Cells	0.1 cc. Washed Cells
1:1			
1:2			
1:4			
1:8			

Procedure for Enzyme Treatment of Red Cells:

All cells are washed four times in normal saline, packed and then divided in two parts; one part is reconstituted as a 2 per cent saline suspension and used as untreated cells for the saline agglutination titration, the other part treated with trypsin[8] or papain and used for comparative titration in saline and by the indirect antiglobulin method.

1. To nine parts of four times washed packed red cells,
2. Add one part of a 1 per cent trypsin (Difco) in standard phosphate buffer solution.
3. Mix well, place in water bath at 37° C. for one hour.
4. Wash three times with normal saline.
5. Dilute packed cells in saline to make a 2 per cent suspension.

Beyond the end point of a titration, which is the highest dilution at which clumping is observed there are usually some cells which though coated, do not form clumps, and coating of these unclumped cells can be detected by the antiglobulin test.

The antiglobulin test is carried out on the cell sediment in each tube of the saline agglutination titration and trypsinated cell titration where no clumping was observed at the end of the period of incubation. The

cells are washed four times and two drops of antihuman globulin serum added and mixed with the cells.

Comment: Trypsinization causes antigens Rho, rh', rh", hr', and hr", to become somewhat more readily coated not only by bivalent antibodies, but also to a greater degree by univalent antibodies. This is also true for the antigen receptors in cases with auto-antibodies of the "warm type." The trypsinated cell antiglobulin method carried out in the test tube and using a high titered antiglobulin serum yields titers equal to or higher than other methods.

M and N factors are largely destroyed by enzyme treatment of red cells[9]. Trypsin treatment neither affects the sensitivity of the Kell antigen nor its ability to become coated with specific antibodies. Kidd antigen is affected in the same fashion as the Kell. In spite of the fact that trypsinated Kell positive cells are coated with univalent antibodies, they fail to clump when saline suspended. In these two respects the Kell factor is affected differently by trypsin than the Rho factor. The Duffy factor seems to be completely destroyed and unable to react with its specific antibody.

Although trypsinization of erythrocytes affects antigens differently, it makes certain antigens more sensitive and permits detection of antibodies specific for them when present in low titer. The method yields sharp and more easily read end points, and eliminates the prozone effect found with some serums.

A, B, P, S, Le^a, Cellano, and Lw^2 factors are made slightly more sensitive or unaffected by trypsinization.

Cold Agglutinins

Procedure: Venous blood is allowed to clot at room temperature or at 37° C. The clear serum is then separated and removed after centrifugation. Tests are carried out immediately or the serum is stored in stoppered tubes in a refrigerator and the tests performed within a few days. This is an important factor, particularly in sera of low titer since there is a certain amount of deterioration on storage.

The cold agglutination is independent of the blood groups, is optimum at 0 to 5° C. diminishes with rise in temperature, and is no longer demonstrable at 37° C. The erythrocytes in the test are obtained from group "O" donors. Oxalated blood is washed three times in a large volume of saline and a 2 percent suspension of cells in physiological saline is made. Almost all of the tests are carried out with cells that are one to four days old.

1. Serial two-fold dilutions of serum or plasma in saline are made in 0.2 ml. amounts in 10 x 75 mm. test tubes.

2. Equal volumes of a 2 per cent cell suspension are added, the tubes shaken and stored in a refrigerator for two hours or overnight.

3. The tubes are usually placed in a cold water bath before the readings are made. In reading the tubes, the tubes are shaken gently to loosen all of the cells from the bottom of the tube and to allow thorough mixing. The agglutination is graded from 4+ to 1+, the former representing a single hard clump of cells not broken up on mixing while the latter indicates a finely flocullar agglutination, easily visible without magnification.

4. After the readings are made in the cold, the racks of tubes are placed in a water bath at 37° C. for two hours. All the positive agglutination should be completely reversed by this procedure.

Comment: Factors influencing the occurrence and titers of cold agglutinins as reported by Finland et al:[11] Cold agglutinins of 1:40 or higher are found in cases of primary atypical pneumonia of unknown etiology. The first positive results are observed between the seventh and twenty-seventh days but mostly during the second and third week. The maximum titers occur mostly between the eleventh and twenty-fourth days. There are a number of cases in which the maximum was not attained until the fifth week or later. The occurrence of cold agglutinins and the height to which the titers rise is related to the severity of the disease. The higher the patients' temperatures, the greater is the proportion of cases with high cold agglutinin titers. The longer the fever persists the greater is the proportion of cases having high titers.

The thermal range of the cold agglutination reaction has been studied from sera of atypical pneumonia. In general, the higher the titer, the more likely is the agglutination to persist as the temperature is increased above 0° C. In sera with low or moderate titers, the agglutination is completely abolished at room temperature (22 to 25° C.), while those with higher titers in the cold often showed considerable agglutination even at 30 to 35° C. This is true for sera of atypical pneumonia cases with or without anemia as well as in cases of hemolytic anemia without pneumonia.

The titer of cold agglutinins is not affected by heating at 56° C. for one-half hour. Temperatures of 66° C. or higher are necessary to completely remove cold agglutinins from the sera.

The indicator cells which are stored in the original plasma give a higher titer and clearer end points than the cells which are stored as a 2 per cent suspension in saline. Cell suspensions of lower concentration (1 per cent) than the one routinely used may also yield higher titers in some sera in which the cold agglutinin content is low.

Test for Cryoglobulin

Blood is collected in a warm syringe and allowed to clot at 37° C. After centrifugation, the serum is separated from the clot and kept in a refrigerator at 5° C. for several hours or over night. If cryoglobulin is present a small translucent button forms at the bottom of the test tube. This button dissolves on warming in a 37° C. water bath. As the temperature is lowered again a heavy floculant precipitate will appear, settle and form a gel in the test tube butt. Repeated heatings and coolings should not alter these characteristics.

Discussion

The procedures described in this paper have been used successfully in our hospital and in many other laboratories in studying cases of hemolytic anemia. The techniques are not complicated and require very little in the way of equipment that is not already available in most routine departments. There are many additional tests such as elutions, differential absorptions, layering antiglobulin and mixed agglutination of Coombs, acidified serum for detection of "cold" hemolysins, etc., which are suited only for the research laboratory.

The "direct" and "indirect" antiglobulin tests have proven to be the most useful as a screening procedure. The antiglobulin test is a simple and reliable method of distinguishing certain cases of idiopathic acquired hemolytic anemia from other hemolytic processes due to congenital abnormality of the red blood cells, and from hemolytic anemia caused by cold agglutinins, alpha or beta agglutinins, chemicals, burns, etc. A positive antiglobulin test occasionally reflects a hemolytic condition concurrently existing with another systemic disease such as lymphatic leukemia, Hodgkin's disease, recurrent thrombocytopenic purpura, disseminated lupus erythematosis, osteosclerotic anemia, hypoplastic anemia later becoming frankly hemolytic.[7] The indirect antiglobulin test is especially sensitive in detecting the "free" antibody in the serum of severe cases.

Patients with hemolytic anemia frequently receive a large number of blood transfusions. It is important to study their serum for the presence of specific blood group antibodies which may be responsible for transfusion reactions. This can be done by the titration methods and the antiglobulin test using the patient's serum and different cell types that are available in most blood banks.

Within recent years importance has been placed on the finding of cold agglutinins in the patient's serum. From the results of in vitro studies the agglutination of erythrocytes renders them more susceptible to hemolysis when subjected to mechanical stimuli.[12] Certain authors also suggest that cohesion of erythrocytes may lead to their increased mechanical destruction while in motion in the circulation.[13] In patients with atypical pneumonia, particularly during the height of the disease in severe cases, there may be transient reduction in the skin temperature, especially in the extremities. This may occur during chills or as a result of the administration of antipyretics.[11] In patients with marked cold auto-agglutination, there is usually a wide thermal range and significant agglutination may still occur at the temperatures which are attained in the skin of the extremities under such conditions. This might conceivably represent the conditions under which hemolysis might occur.

Significant titers of cold agglutinins are found in cases of primary atypical pneumonia, mumps complicated with orchitis, and occasionally in tropical eosinophilia, and infectious mononucleosis. Cold agglutinins have also been described in many other conditions, notably in association with various blood dyscrasias, liver diseases, and in allergic and peripheral vascular disorders. The occurrence in these conditions is still sufficiently rare that only individual cases or very small groups of cases have been reported. The only infectious disease in which cold auto-agglutination has been found with any regularity is trypanosomiasis.[11]

Cryoglobulinemia has been reported in Kala-azar, subacute bacterial endocarditis, multiple myeloma, and chronic lymphatic leukemia. In a few of these conditions elevated cold agglutinins or hemolytic anemia were present.

The immunohematology procedures described in this report only detect the presence of erythrocyte agglutinins. The mechanisms by which actual lysis takes place in vivo are not clearly known, and probably vary from case to case. Hemolysins may play a role, but our present in-vitro techniques fail to demonstrate them in most serums where there

are auto-agglutinins. Tissue lysins acting upon antibody-coated cells may also be of some importance. Enzymes such as Beta-glucuronidase and hyaluronidase[14] are tissue enzymes which have been proven to be hemolytic for red cells in-vitro. The spleen may function not only as a tissue lytic agent but as a trapping organ for already injured cells, thus causing lysis. Some or all of these may be factors that play a part in the abnormal destruction of the red cells.

The auto-antibodies alone must then be of secondary importance. Evidence in support of this hypothesis is provided by the frequent clinical and hematological remission observed after splenectomy or ACTH/Cortisone therapy in spite of unchanged auto-immunization as estimated by the result of a positive direct Coombs test.

Recent studies, principally survival-time determinations on erythrocytes, have shown that hemolytic syndromes are common as a complication of a large number of diseases, such as leukemias, Hodgkin's disease, reticulum cell sarcoma, generalized cancers, infections, liver and renal disease. In these disorders there are usually no signs of auto-immunization though a few cases have demonstrated positive Coombs test.

Summary

There are certain practical immunohematology techniques such as the antiglobulin test, antibody titrations, enzyme treatment of cells, and cold agglutinations which can give valuable information in some cases of hemolytic anemia. These procedures should be employed in as many laboratories as possible as aids in studying the hemolytic process. It should be pointed out, however, that there are many cases of clinical hemolysis which give completely negative results. Either our present tests are not sensitive enough to demonstrate the presence of an immune erythrocyte antibody or other factors are responsible for the lysis of red cells that require new methods of detection.

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AMONG THE NEW BOOKS

SEIVERD'S HEMATOLOGY FOR THE MEDICAL TECHNOLOGIST. Second Edition by Charles E. Seiverd, Chief Technologist, Doctor's Clinical Laboratory, Director of Research, The Horizon Laboratories, Glendale, Arizona. Lea & Febiger, Philadelphia, Pa. 1958. 275 pages. 76 illustrations, 13 colored plates. $5.75.

Decidedly a volume designed from a practical viewpoint for the medical technologist and the student in medical technology, this gives in detail the techniques often taken for granted. In several instances alternative methods are given. There is much material here in one small book.

BIOCHEMISTRY OF SOME PEPTIDE AND STEROID ANTIBIOTICS. by E. P. Abraham, M.A., Ph.D. (Oxford), John Wiley & Sons, Inc., Publishers, New York, N. Y. 1957. 96 pp. 9 illustrations, 6 tables, Charts. $3.00.

One of the annual series of Ciba Lectures in Microbial Biochemistry comprise this book. The lectures on the *Bacitracins*, the *Cephalosporins*, and their *Structural and Functional Relationships* will appeal more to the researcher than to the clinical laboratory technologist.

THE CHEMISTRY OF THE STEROIDS. By W. Klyne, M.A., D. Sc., Ph.D., Reader in Biochemistry University of London (Postgraduate Medical School). Methuen & Co., Ltd., London. John Wiley & Sons, Inc., New York. 1957. 216 pp. 30 tables. 3 illustrations. Formulae. $3.50.

While the author, in his preface, apologizes for "one or two" very "elementary" sections, it must be understood that just such material as this will enable the biologist better to understand the chemist's point of view in the field related to steroids. There are 16 pages of references.

CURRENTS IN BIOCHEMICAL RESEARCH, 1956. Edited by David E. Green, Institute for Enzyme Research, University of Wisconsin, Madison. Interscience Publishers, New York & London. 1956. 713 pp. 69 illustrations. 29 tables. $10.00.

There are some 27 contributors to this wealth of material for the chemical researcher and medical technologist, alike. Stressing the objectives "to communicate to non-specialists an overall impression of the significant problems in each field," as well as to "point up the broad strategy of current research," this volume is more than adequate. It will be used for reference to evaluate the present status of these major fields.

Announcing, the publication in June of the third annual volume of the **Bibliography of Medical Reviews.**

Review articles listed in Volume 1 and 2 were gathered as a by-product of the *Current List of Medical Literature* operation and were duplicated in the parent publication in another format. With Volume 3, however, the collection of review articles was extended to all of the current journals received by the National Library of Medicine. The result has been the inclusion n Volume 3 of approximately 600 non-*Current List* articles along with the 2300 review articles also listed in the *Current List.*

The 1958 volume of the *Bibliography of Medical Reviews* is arranged by subject with a separate author index and will contain approximately 2900 references to review articles in clinical and experimental medicine and allied fields which have appeared largely in 1957. Copies of Volume 3 for 1958 will be available from the Superintendent of Documents, U.S. Government Printing Office, Washington 25, D. C., at a price presently estimated at $1.25.

A NOMOGRAM FOR THE RAPID CALCULATION OF TUBULAR REABSORPTION OF PHOSPHORUS IN HYPERPARATHYROIDISM*†

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Although hypercalcemia is the most reliable diagnostic criterion for hyperparathyroidism, it is not pathognomonic of the condition and in some patients with borderline elevations of the calcium level further laboratory procedures are necessary. In 1956 Chambers, Gordan, Goldman and Reifenstein[3] reported their evaluation of several tests for hyperparathyroidism and emphasized the usefulness of the tubular reabsorption of phosphate (TRP) in the diagnosis of hyperparathyroidism in patients without renal insufficiency. Since parathyroid hormone causes a decreased reabsorption of phosphate in the kidney tubules, it was suggested that parathyroid function might be measured by the determination of the percentage of phosphorus filtered by the glomeruli which is reabsorbed by the renal tubules.

It is the purpose of this paper to present a nomogram[4] (Figure 1) which simplifies the calculation of the tubular reabsorption of phosphorus (TRP) based on the following formula of Nordin and Fraser.[7]

$$\%\text{TRP} = 100\left[1 - \frac{\text{urine phosphorus}}{\text{urine creatinine}} \times \frac{\text{serum creatinine}}{\text{serum phosphorus}}\right]$$

Since only the relatively common chemical tests for phosphorus and creatinine are required, the determination of TRP is readily adaptable to most clinical laboratories. Although the original authors[3] refer to the determination of phosphates, the actual laboratory analysis is for inorganic phosphorus, which was determined in our laboratory by the method of Simonsen, Wertman, Westover and Mehl.[8] Creatinine levels were determined by the method of Bonsnes and Taussky,[1] and only those patients with a serum creatinine level of 1.3 mg% or less were considered to be without renal insufficiency. Serum calcium levels were determined by chelation using the method of Fales[5] as modified by Buckner and Shively.[2]

The normal values as presented by Chambers, Gordan, Goldman and Reifenstein[3] established by 66 control studies on 53 subjects and 40 studies on 10 patients, are as follows:

	%TRP
Normal	78 - 91 (Mean 84.3)
Hyperparathyroidism	41 - 82 (Mean 65.0)

In the study of individuals from families with hereditary hyperparathyroidism,[6] we found the TRP to be helpful not only in establishing the diagnosis of hyperparathyroidism but also in the evaluation of the completeness of removal of hyperfunctioning parathyroid tissue.

Summary

A nomogram for the rapid calculation of the tubular reabsorption of phosphorus (TRP) is presented.

* Sponsored by the Caylor-Nickel Research Foundation.
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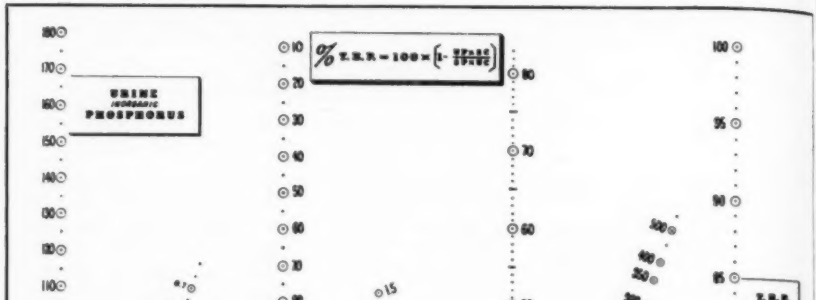

Figure 1

Nomographic chart for determining tubular reabsorption of phosphorus. With a straight edge, preferably a transparent ruler, connect the urine phosphorus value with the serum creatinine value to a point of intersection on scale A. This point connected with the serum phosphorus value gives a level on scale B which connected to the urinary creatinine value indicates the %TRP on the final scale.

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UNUSUAL CASES REVEALED IN ROUTINE BLOOD AND RH STUDIES*

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Many surveys have been made relative to the ABO groups and the incidence in which they occur.[1,2] The discovery of the Rh factor brought about additional immunohematological studies and procedures. The related Rh-hr system, and the Du factor have also increased the complexity of the routine tests.

It is the object of this paper to (1) present the statistical data obtained from routine studies of pregnant women admitted to Lafayette Charity Hospital from 1953 to 1957; (2) relate this data to the incidence of blood groups and Rh factors in the white and colored races; (3) discuss the probable causes of sensitization in these women, whether due to previous pregnancies or blood transfusions, and (4) present some interesting facts found in the routine testing of donors.

The obstetrical department of this hospital averages 4,025 deliveries yearly, of which 79% are of the colored race. We, in the Blood Bank, have the opportunity of doing serological and immunological studies on these cases. Some of the patients included in this present study attended our prenatal clinic or the State Health Clinic held in their respective parishes while others had little or no prenatal care and our work was not done until the time of delivery.

Our Bank also processes blood from approximately 4,000 donors yearly, and a report of unusual findings on two donors is given. Some of these case-history studies will clarify the necessity of giving blood compatible for the factors C and E, as well as for the D factor to all females who are within or under the child bearing age. It is also advisable to do a routine check for the Du factor on Rh negative donors.

Materials and Procedures

During the period covered by this study our routine prenatal work consisted of ABO grouping and determination of the Rh_0 or D factor. When we found the patient to be Rh negative, we carried out complete Rh-hr studies on her, also on her husband when he was available. Commercially prepared sera that met or exceeded specifications of the National Institutes of Health were used. Routine screening tests for sensitization were done on Rh negative women. In studying these, we used the albumin method with a panel of selected Group O CDE cells or cells from the patient's husband. In addition trypsinized cells were used on some, and all were checked by the Indirect Coombs method, using anti-human globulin. We were able to observe the antibody titers at given intervals on some of the sensitized women.

The blood group was established on donors by using commercially prepared sera, and, also the reverse grouping by using the donor's serum with known A B & O cells. The Rh classification was established by the slide and modified test tube method, using Anti-D Serum. Rh-negative donors were further studied for the Du, C and E factors.

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* Third SPF Award, Blood Bank, 1957. Read before 25th Annual ASMT Convention, Chicago, Illinois, June 1957.

and a screening test was done for saline agglutinins and blocking antibodies.

Analysis of Data

The series in the first chart represents a total of 8,136 cases. After our work was established on a routine basis in 1953, we obtained a sample of blood from each patient on her first visit to the prenatal clinics, or upon admission to our hospital. In this chart we are concerned with the numbers of cde, Cde, cdE, and CdE women as they fall in the white and colored races. This work parallels other statistics relative to the percentage of Rh negatives in each race; however, our data differs slightly in frequency of combinations, namely CdE.

Chart number two indicates a portion of our study on 752 Rh negative women. Here we correlate the Blood Groups A, B, O, and AB with sensitization and non-sensitization in the women, and, also the frequency with which the various Rh combinations fall in the ABO groups.

CHART #1

8,136 Obstetrical Cases

	# OB Cases	cde	Cde	cdE	CdE
White	2,516	342	28	11	1
Colored	5,620	309	50	8	3

CHART #2

752 Rh Negative Women

	Sensitized	Non Sensitized	cde	Cde	cdE	CdE
A	23	249	241	22	7	2
B	6	98	80	20	3	1
O	16	330	302	35	8	1
AB	1	29	28	1	1	..

In chart number three we are handling 46 selected sensitized women, who have had 288 pregnancies with 292 babies. Also listed is the number of times we found antibodies and the pregnancy in which they were first detected. A few of the women in this series have records in our hospital dating back to 1938, at which time the Rh factor was not a known problem in blood transfusion. In each of these women blood was given prior to the pregnancy during which sensitization was first detected.

For example, one woman belonging to Group O cde did not have antibodies at the time of her fourteenth delivery. She received 500 c.c. of type specific Rh negative (cde) blood during this delivery. When this woman returned to the prenatal clinic during her fifteenth pregnancy, she was found to possess blocking or incomplete antibodies. She had the same husband for the past ten pregnancies, including the fifteenth. In checking our Blood Bank records, we were able to identify the donor. Repeat tests on this donor indicated he was Rh negative (cde), but further testing revealed so-called "Rh negative" to be Du positive.

CHART #3

	Gravida X	Number of Women	Non-Sensitized	Incomplete Antibodies	Complete Antibodies	Hist. of Trans. with Rh Pos. or Rh Unknown Blood	Normal Babies	Erythroblastosis Fetalis Recovered	Stillbirths	Neonatal Deaths	Autopsies of Stillbirths and Neonatal Deaths
Column	1	2	3	4	5	6	7	8	9	10	11
	1	46	44	2	1	2	32	1	12	1	4
	2	46	40	6	..	2	31	4	11	..	3
	3	39	26	13	2	..	22	7	6	4	2
	4	33	24	9	2	2	24	4	3	2	..
	5	31	14	17	1	1	14	12	2	3	1
	6	24	10	14	1	1	10	10	2	2	..
	7	22	7	15	1	..	8	11	3	1	1
	8	12	6	6	1	1	6	3	2	1	..
	9	10	6	4	1	1	6	5	1	..	..
	10	7	6	1	..	..	5	1	1	..	..
	11	5	4	1	..	1	4	2	..	..	..
	12	5	2	3	1	..	2	2	1	..	..
	13	3	1	2	..	1	1	1	1	..	..
	14	3	1	2	1	1	1	1	1	..	1
	15	2	..	2	..	..	..	1	..	1	..

Columns 1 and 2 on this third chart show the number of women and how many pregnancies each had. Columns 3, 4, and 5 differentiate the women into sensitized and nonsensitized categories, and indicate whether complete and/or incomplete antibodies were present. In checking these with column #1, one can readily see in which pregnancy antibodies were first detected. Column #6 states the number of women with histories of transfusion with Rh positive, or Rh unknown bloods. Column #7 shows the number of normal babies and #8 the number having recovered from erythroblastosis fetalis. Column #9 reveals the number of stillbirths, and #10 the neonatal deaths.

Let us take a cross section of this third chart. The first pregnancies included forty-six women of whom forty-four were found to be nonsensitized, one sensitized and possessing incomplete and complete antibodies, and one incomplete antibodies only. The two sensitized women gave histories of transfusions with group specific blood of unknown Rh factor. Data on the babies included thirty-two normal, one erythroblastotic with recovery, twelve stillbirths, and one neonatal death. The number of women dropped to thirty-nine on third pregnancy, with thirteen showing sensitization. The data on third pregnancies included twenty-two normal babies, seven showing evidence of hemolytic disease of the newborn, six stillbirths, and four neonatal deaths. Another shift was noted in fifth pregnancies where the number of women dropped to 31. Then at the sixth pregnancy another drop was noted in the number of women with, however, a steady increase in the percentage of those

being sensitized and an increase in the number of abnormal babies. Through the courtesy of the Department of Pathology we were able to chart the number of autopsies done on stillbirth and neonatal deaths.

Within the past eighteen months we have noticed an apparently marked increase in the number of babies with hemolytic disease of the newborn due to ABO incompatibility.

When the confirmation blood group was done using the donor serum at room temperature, only anti-A was demonstrable in two donors whose cells had been previously grouped as O. The tests were repeated and only at a lower temperature (18° C.) did anti-B become evident. No atypical antibodies were found in the serum of these donors.

Case Histories and Clinical Data

Case #1, a white female, group O Rh positive (CDe/c) gave a history of three well and normal children, with no interrupted pregnancies. A transfusion of Group O Rh positive blood was given at the time of her third delivery. The fourth baby showed clinical symptoms of erythroblastosis fetalis. Tests on the baby's blood showed her to be Group O Rh positive (CDE/c), the Direct Coombs positive. The serum of the mother, when tested with a panel of selected cells, showed antibodies against E with a titer of 1:64 and also antibodies against Kell.

Case #2, a white female, Group O Rh positive (CDE/c) gave a history of her first three pregnancies terminating in spontaneous abortions at six weeks gestation. The causes were unknown. Her fourth, fifth, and sixth pregnancies were normal. The seventh baby was slightly jaundiced at birth but cleared up within a few days without specific medications. The eighth child was delivered in our hospital on September 3, 1954. At six hours the baby became jaundiced. The hemoglobin was 19.0 grams, bilirubin 4.2 milligrams per cent, and the Direct Coombs positive (weakly). The baby's blood proved to be Group A Rh positive (CDE/c) and that of the father Group A cde/c. Tests on the mother's serum using a panel of selected cells were entirely negative for atypical antibodies. Upon titration we found titers of 1:80 and 1:40 of anti-A and anti-B slightly "immune." At twenty-four hours the hemoglobin showed a marked decline while the bilirubin showed a sharp rise. A replacement transfusion was given using low titer Group O Rh positive blood to which 10.0 c.c. of Group Specific Substance A and B had been added. Kernicterus developed at forty-eight hours, and the baby died at seventy-two hours. An autopsy confirmed the diagnosis of erythroblastosis fetalis. During this woman's ninth pregnancy, she was attended by a private physician. However, samples of her blood were sent to us at six months gestation. Titration at this time showed values of 1:160 and 1:80 for anti-A and anti-B respectively. After partial neutralization both anti-A and anti-B were found to be of the "immune" variety. No further prenatal work was done on this woman by us. She delivered in another hospital at nine months gestation. Blood on the newborn was sent to us and proved to be Group A Rh negative (cde), Direct Coombs negative. The diagnosis, treatment, and results were the same as for her eighth child. Incidentally her children from the fourth, fifth, sixth, and seventh pregnancies were all Group A.

Case #3, a white female, Group A Rh positive gave a history of no

blood transfusions or interrupted pregnancies. Her first six children were normal and well, but her seventh baby showed jaundice at twelve hours. Detailed studies on the baby, mother, and father showed the baby to be Group A CDE/c, mother Group A CDE/C and father Group A CDE/c. The serum of the mother proved to have blocking antibodies against the hr′(c) factor. The baby received one replacement plus several fractional transfusions. The child is well and normal at 2½ years of age. This patient is now pregnant for the eighth time. Tests showed her to be free of hr′ antibodies at three months gestation, on February 15, 1957.

Discussion

We can probably attribute antibody stimulation to blood transfusions in twelve of the women studied. Some definitely were the recipients of Rh positive or Du positive blood, while others received blood of undetermined Rh classification.

We must not attribute all of the stillbirths recorded to Rh sensitization. Some can be attributed to ABO incompatibility, some to little or no prenatal care, and some to syphilis (3.7% of the colored women attending the prenatal clinics were found to have a positive VDRL). Note the majority of the stillbirths occurred during the first few pregnancies when the number of sensitized mothers was still low.

The apparently marked increase in the number of reported cases of erythroblastosis due to ABO incompatibility may be due in part to an increased awareness on the part of our pediatricians. This increase, then, could be due to an increase in the number of times the disease is diagnosed, rather than to an actual increase in incidence. Also, the marked increase in the use of blood as a therapeutic measure may have some significance. We have just started keeping more complete records on those mothers found to have "immune" anti-A and B causing mild cases of ABO erythroblastosis.

All females of child bearing age or under should receive blood compatible for the C, E and c factors as well as for the D.

It is most important that all Rh negative (cde) donors also be proved Du negative.

Summary

Statistical data, correlating the white and colored races relative to the Rh factor has been presented. A report on 752 Rh negative women, showing sensitization and nonsensitization, and the incidence of the various Rh combinations in the ABO categories has also been included.

A selection of forty-six sensitized women who had a combined number of 288 pregnancies with 292 babies was charted. The chart reveals in which pregnancy antibody was first detected. The fact was established that previous blood transfusions were probably the cause of antibody stimulation in some of the cases. The number of normal babies and those recovering from erythroblastosis fetalis has been listed, also the number of stillbirths and neonatal deaths.

One case with repeated occurrences of fatal erythroblastosis fetalis caused by ABO incompatibility was discussed. Also a case of sensitization to the E and Kell factors, and another case of sensitization to hr′

(c) were mentioned. Facts were established that one woman became sensitized by receiving a transfusion of Du positive blood.

Temperature proved to be the critical factor when doing reverse or confirmation grouping on two donors who had been previously grouped as O.

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CORRECTION

March-April 1958, article entitled "*Atypical* Acid-Fast Bacilli" by Shirley McMillen. Atypical *not* A Typical. In line 10, same article, *cocco-bacillary*.

In the article, "Virulence Tests for Mycobacteria," by Yosh Takimura, under authorship, laboratories, *not* labarotories.

CURRENT STATUS OF THE MT (ASCP) WITH RELATIONSHIP TO WAGES AND HOURS, UNIONIZATION, LICENSURE AND PROFESSIONAL RECOGNITION*

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Introduction

Two years ago there was a terrific explosion in a hardware store in Roselle which made headlines all over the country. The explosion was due to an accumulation of gas when utility workers pierced a gas line while installing a street light. It had far-reaching effects on those involved—a huge financial responsibility for the utility companies and tragedy in the families of the dead and injured.

Laboratory personnel, be it ASCP registered or otherwise, is handling ingredients which unless treated with care could be explosive. These ingredients assigned to me as a member of this panel discussion are

1) working conditions (wages and hours)
2) union activities
3) licensure
4) professional recognition

The material which I shall present has been collected from information that has come to me from committee members, state journals and other publications through my work on the ASMT public relations committee.

We all recognize the sad fact that there is a great shortage of medical technologists and that the reason for this is INSUFFICIENT (and possibly—INEFFICIENT) recruiting. Possibly those who are trying to recruit are square pegs in round holes and aptitude testing as described by Dr. Hepler just a few minutes ago might not be a bad idea for our recruitment speakers. The two main factors influencing this lack of recruitment of trainees are (1) low salaries and (2) lack of professional status.

I. SALARIES AND HOURS

Let's take a look at the salary and hours situation and its possible solution, first realizing that any comparative study must be much more comprehensive than anything presented here because of differences in geographical distribution, types of employment (hospital, private office, industrial, etc.) and other factors.

A. Current Status: What is the current status of salaries and hours?

1. U. S. Department of Labor:[1] In its statistics for 1956-57 the U. S. Department of Labor reported on the average weekly salaries for women medical technologists. This report showed the highest average salaries in California and the lowest in New England. In other words the old adage of "Go West, young man" might well be changed to include young women as well.

According to this survey, Chicago salaries would approximate $305.00 a month.

2. **Report of the Survey of Medical Technologists:** In December 1953 a survey of medical technologists was authorized and supported by the

* Presented at the Spring Seminar, IMTA, March, 1958, Danville, Illinois.

executive committee of ASCP under the auspices of the Board of Registry of Medical Technologists and the supervision of Dr. J. L. Arbogast. From all the questionnaires sent out only the general MT certificate group (6,724) was included for the tabulated report. Many very interesting conclusions could be drawn from the results. As for the salaries and hours the following facts may be of interest:

	Monthly Salary	Hours per Week
Average (6,724)	250–299	40
Regional		
Illinois	300–325	
Maine (lowest)	175–200	

3. **Illinois Hospital Association**:[2] The 1957 survey of hospital salaries made by the Illinois Hospital Association includes 18 classifications. It reports comparisons of salaries of all classifications as well as other data. Only cash amounts paid are covered. The average "going" salaries and working hours of all districts in the state are as follows:

	Monthly Salary	Hours/Week
* MT (ASCP)	$371	40.6
Non-registered	301	40.5

B. **Reasons for Changing Jobs**: Interesting data in the survey authorized by Dr. Arbogast were the reasons given for changing jobs in order of frequency:

1. family convenience,
2. better working conditions,
3. better pay.

C. **Actions Being Taken**: What are other states doing about the working conditions of the medical technologists?

1. **Colorado**: Dr. Catherine Anthony, Advisor to the Denver Society of Medical Technologists, reported on a meeting of pathologists and administrators where, among other matters, the technologists—their recognition and pay scale—were discussed.

A committee headed by Dr. Eugene Hildebrand, Pathologist, and a Colorado administrator recommended a pay scale to be adopted for the medical technologists starting on September 1, 1957 with the following sliding scale:

	Starting salary	1 year	3 years
Unregistered	250	275	300
Registered—no degree)	300	325	350
Degree—not registered)			
ASCP registered and degree	325	350	375
Department supervisors	Add $25 to all classifications		
Chief technologist—	Minimum $450		

Many hospitals have already complied.

2. **Washington State**: Following the lead of the Detroit Society, the Tacoma Society members and five members of the executive committee

*(Includes a high of $725 found in the Peoria district)

of WSSMT prepared recommendations as to minimum employment regulations to keep medical technologists in the field and not shifting.

Aide	200-250
Non-registered) eligible) experienced)	300-350
Jr. MT (registered less than 2 years)	350-400
Sr. MT	
registered 2-5 years	400-450
registered more than 5 years	450-550
Supervisors	25-100 plus specific classification

Call time—$3.00 per night stand-by-time, time and a half when called.

Vacations—2 weeks after 1 year
3 weeks after 3 years
4 weeks after 7 years or more

Sick leave

Copies were sent to all pathologists requesting that they be discussed with the hospital administrators.

3. **Michigan** (Detroit): The preliminary report of the Detroit Society of medical technologists' study committee for the investigation of the laboratory problem was presented at a meeting of the Michigan Pathologists. The survey was taken by the technologists because of lack of response to recruitment efforts and consisted only of four hospitals within the Detroit area.

The Detroit Society made the following proposals:

a. that the pay be commensurate with training and ability;
b. that the training and ability of the ASCP registered technologist be utilized freeing them from tasks requiring no specific training;
c. that there be pathologist-technologist conferences;
d. that the following pay scale be adopted;

Aide	250-300
Non-registered eligible Army-Navy	300-400
Jr. MT (registered less than 1 year)	400-450
Sr. MT	
Registered 1-5 years	450-550
Registered 5-10 years	600

Supervisors—25-100 more than specific classification.

4. **New York** (Empire State Association): Because of the activity of the unions the Empire State Association has set up a committee on economics with regional representatives. This committee's activities thus far are:

a. sponsorship of recommendation of meetings with all hospital chief technologists to
   i. arrive at practical minimum salaries for all laboratory workers,
   ii. discuss present working conditions,
   iii. discuss pros and cons of unions

iv. discuss need to improve professional status and enhance recruitment;
b. mailing of "Salary Survey" to all laboratory personnel;
c. request for meeting with representatives of the hospital administrators for possible joint action to solve some of the economic problems.

These are reports from just four state societies. Others are probably working along similar lines.

II. UNIONIZATION

At the ASMT House of Delegates in Chicago a report was read about union activity in Washington, D. C., with its intention of extending its activities to New York, Chicago and Detroit. This report dealt with the Federation of Technical Medical Personnel AFL-CIO which is chartered by the Office Employees International Union.[3]

Some of the reasons why the ASCP registered technologists do not even consider membership in this union are:

1. incompatibility with our code of ethics;
2. strength of the union in the strike;
3. prime interest of the union—economics (dues of $5.00 monthly with a possible $250 a year)
4. placement of our profession under obligation to a sponsoring organization with no understanding of our responsibilities.

A. **Union Activity in Illinois:** The only activity which has come to our attention thus far is that at the University of Illinois Medical Center in Chicago. There are three schools at the university—pharmacy, dentistry and medical—which employ all types of laboratory personnel.

A meeting was called. Only those interested attended. The registered medical technologists were not interested in the union and did not go. When a vote was called those attending the meeting voted "Yes" for an open shop.

The intimation is that this particular organization may die of disinterest as it is almost impossible to affect the salary situation in civil service institutions.

If you hear of any union activity, **attend** the meetings, vote and report to your society.

III. LICENSURE

This problem must be considered thoughtfully and carefully—it may be disastrous if hastily studied and planned.

What is the **purpose** of state licensure? **Protection** of the public.

A. **Current Status in ASMT Constituent Societies:** There are three constituent societies in ASMT that have state licensure at the present time —California, Florida and Alabama. Many states are on the fence and are anxious to listen to the legislation conference at the 1958 convention.

Bills are being introduced in a number of states by other organizations and are being carefully reviewed for action. Four bills have been introduced in New York State none of them by our affiliated society. These bills are not good and through vigilance and action are expected to be shelved or killed.

B. **Suggested Pros and Cons:** A special mailing will go to each member

listing a number of pros and cons with comments that have been published in other state media, but a few will be stated at this time:

1. **Pros**: (with the assumption that a bill for licensure would be **good.**)
   a. Would protect the Public Health by assuring laboratory work of definite standards.
   b. Would provide legal definition of the profession: no definition at the present—each court can set own interpretation.
   c. Would give positive control of schools of medical technology—schools would be forced to meet certain minimum requirements (inspection by state inspectors, requirement for graduates to pass state examinations).
2. **Cons**:
   a. Unnecessary duplication of authority, function and requirements of the Registry. Registry is voluntary, licensure is mandatory.
   b. Difficulty and cost of preparing a good bill with subsequent licensure examination and renewal cost to the technologist.
   c. Lack of reciprocity between states. The nursing and medical professions are working for recognition of national certification to simplify reciprocity which they do not enjoy at present.

C. **Actions Being Taken**

1. **Constituent Societies**
   a. **Massachusetts**—licensing of approved schools
   b. **Tennessee**—liaison committee of four pathologists and four medical technologists to study (i) recruitment and (ii) state licensure.
   c. **Missouri**—recommendations of its Board of Directors at fall meeting, 1957 that the society go on record as
      i. opposed to unionization of the medical technologists;
      ii. opposed to any idea of creating a new independent certifying board causing a separation from ASCP;
      iii. opposed to state licensure in any form.

2. **ASMT Legislation Committee**: This committee is currently working on the following problems to aid the constituent societies:
   a. remodeled model bill;
   b. proposed standards for the evaluation of state licensure;
   c. pros and cons of state licensure;
   d. panel discussion on legislative subjects at the 1958 convention;
   e. methods of dealing with labor organization problems;
   f. methods of obtaining acceptable legislation.

IV. PROFESSIONAL STATUS

As individual constituent societies and as members of ASMT we are progressing rapidly in professional recognition because of our good public relations in affiliations with other professional organizations.

A. **Illinois Medical Technologists Association**: membership or representation in the following:

1. Tri-State Hospital Assembly
2. Illinois Academy of Science
3. Illinois Hospital Association—Committee on Paramedical Services
4. Illinois Statewide Committee for Public Health.

B. **ASMT**: membership or representation in the following:

1. National Health Council
2. National Committee for Careers in Medical Technology
3. Intersociety Committee on Laboratory Services
4. American Association for Advancement of Science
5. American Association of Blood Banking
6. Board of Registry
7. Board of Schools of Medical Technology
8. Medical Technology Study Committee

SUMMARY

To summarize—you have heard a presentation on four controversial subjects affecting laboratory personnel:

1. Salaries and Hours—present status and actions being taken;
2. Union Activities—present status and actions being taken;
3. State Licensure—present status, some pros and cons, actions being taken by the constituent societies and by the ASMT legislation committee;
4. Professional Status—IMTA and ASMT.

CONCLUSION

In conclusion—should we not

1. recognize the need of all types of workers in our field be they lab aides, junior or senior technologists, instructors or chief technologists;
2. continue to give thought and study to the problems of working conditions and state licensure;
3. keep constantly alert to any union activities;
4. work and cooperate with our pathologists and hospital administrators on these mutual problems;
5. be guided on these particular issues by the concentrated studies and reports of our state and national legislation and standards and studies committees?

By our action on these subjects, we will affect not only ourselves but the pathologists, the hospital administrators and the public.

Our enthusiasm and zeal, possible sense of injustice and dissatisfaction (or what have you?) may lead to an "explosion." To avoid any unknown by-products of an explosive reaction, let's handle our problems carefully and wisely.

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EVALUATION OF STAINING PROCEDURES FOR SEX CELL DETERMINATION IN PERIPHERAL BLOOD SMEARS*

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Introduction

In 1954, William Davidson and Robertson Smith published the results of their research involving sex determination using peripheral blood smears. In their work they described the chromatin nodule or "drumstick" which appears characteristically in the polymorphonuclear leukocytes in peripheral blood smears of females in a ratio of at least 6 cells containing the structure in 500 neutrophiles counted. These structures have a homogenous, rounded, solid head, and are joined to the nucleus of the polymorphonuclear leukocyte by a single, fine chromatin thread. The head measures approximately 1.5_u[1] in diameter. In the blood smear of the male the true drumstick is never found. Several structures may be confused with this drumstick, however. Among these are small clubs, less than 1_u in diameter;[2] minor lobes; and racquets, having a pale central area in the head. The quality of the smear, therefore, and the preparation with which it is stained are of the greatest importance if accurate results are to be obtained.

In the articles which have been written to date concerning this work, no methods for the processing of the smears have been given. Only the results of the drumstick tabulations are discussed in most of these with a passing mention of the stain used. Therefore, since the method of preparation of the slides is of such consequence it is the purpose of this paper to present and discuss a number of staining techniques which would be readily available to most laboratories and to evaluate the results of these stains.

Methods

In order to become acquainted with the structural characteristics of the drumstick as well as the nuclear projections which may be confused with it, 20 random slides were studied. These were routine smears which came into the laboratory. They represented both sexes. These were studied until the typical drumstick became easily recognized and familiar. Ten smears were then studied in which the sex was unknown at the time of the examination. These were identified with 100 per cent accuracy.

The next step was to determine the type of smear best suited to the study of these features. It was decided almost immediately that a fairly thin smear gave the best results. These smears should cover the slide almost completely in order to allow for tabulation of drumsticks in low leukocyte counts. Fresh venous blood is necessary for best results. Oxalating the blood causes undesirable changes in the leukocytes which add to the difficulty in identifying the drumsticks.

When both the structure of the drumstick and its many characteristics had become familiar and the most desirable type of smear had been decided upon, a number of stains were studied in an effort to find the one giving the best results. The following data give the five stains and the staining methods used in this investigation.

* Read on program 25th ASMT Convention, Chicago, Illinois, June, 1957.

A. *Wright's Stain*

The preparation and procedure for this stain may be found in any comprehensive text book. It must be mentioned here, however, that it is important for the purpose of sex determination that the stain be carefully filtered. Precipitated stain adds obvious difficulties of the study of the blood smears.

B. *Giemsa Stain*

Preparation: The commercially prepared stain is mixed in a proportion of 0.1cc per 1cc of distilled water.

Procedure: Fix the slides in methyl alcohol for 3 to 5 minutes. Air-dry them. Cover with Giemsa stain for 30 minutes. Wash with distilled water.

C. *Wright-Giemsa Stain*

Preparation: See above instructions concerning each of these stains.

Procedure: Cover the smear with Wright's stain for 3 minutes. Pour off the stain. Cover with Giemsa for a counterstain for 30 minutes. Wash with distilled water.

D. *Supravital Brilliant Cresyl Blue-Giemsa Stain*

Preparation of slides: Prepare the whole surface of the slides which have been previously cleaned with methyl alcohol. The slides are prepared by placing them in a staining dish and pouring a 1 per cent solution of alcoholic Brilliant Cresyl Blue over them, and then draining them. They will have a slight greyish-violet appearance.

Procedure: Smear the blood carefully on the dry slide prepared as above stated. Place the slide in a damp chamber for 5 to 10 minutes. A Petri dish with a wet filter paper lining is ideal for this purpose. Air-dry the slide thoroughly. Fix with methyl alcohol for 3 to 5 minutes. Counterstain with Giemsa for 30 minutes.[3]

E. *May-Grunwald-Giemsa Stain (Modified Pappenheim)*

Preparation: Make a saturated solution of May-Grunwald stain in absolute alcohol. This requires approximately 6 grams per 100 cc of alcohol.

Procedure: Fix the air-dried smears by immersing in a dish of filtered May-Grunwald stain for 5 minutes. This solution can be used several times if it is protected from moisture. Rinse with distilled water and air-dry the smear. When dry, stain with Giemsa for 30 minutes and wash with distilled water.[4]

Results

After the staining procedures had become familiar, five apparently healthy females were chosen as controls. Their leukocyte counts ranged from 6,000 to 12,000. The drumstick tabulations on the group ranged from 6 in 127 to 6 in 300. Venous blood was obtained from each of these and a set of 5 blood smears was made. Each smear was stained by a different method as mentioned above. Since each set of smears was obtained and stained separately from the others and no mass staining was used, each stain was tested five times. The smears were studied and evaluated individually. The results of this evaluation are listed in the included chart.

EVALUATION OF STAINING PROCEDURES FOR SEX CELL DETERMINATION

Controls	WBC	Drumstick Tabulation	Wright's	Giemsa	Wright-Giemsa	Supravital-Giemsa	May-Grunwald-Giemsa
#1.....	6,000	6/223	Stain clear. Identification not difficult.	Too pale for identification of any cellular elements.	Very clear. Accurate identification possible.	Very pale. No elements identifiable.	Very clear. Nuclear and chromosomal elements easily identifiable.
#2.....	7,400	6/185	Stain pale but identification not difficult.	Very pale. No identification possible.	Clear stain. Accurate identification possible.	Pale stain. Cellular elements distorted.	Cellular elements fairly clear.
#3.....	9,400	6/275	Stain precipitated. Drumsticks hard to see.	Very pale. No identification possible.	Very clear. Accurate identification possible.	Very pale. Identification impossible.	Very clear stain. Cellular elements easily seen.
#4.....	12,000	6/320	Stain clear. Identification not difficult.	Very pale. No identification possible.	Very clear stain. Identification not difficult.	Very pale. Identification impossible.	Very clear. Nuclear and cellular elements easily identifiable.
#5.....	10,800	6/177	Clear stain. Identification not difficult.	Very pale. No identification possible.	Very clear. stain. Accurate identification possible.	Very pale. Cellualr elements distorted.	Very clear. Nuclear and chromosomal elements easily identifiable.

Discussion

The results of the comparison of the five stains left no doubt that either a Wright's stain or a May-Grunwald stain when counterstained with Giemsa bring out the drumstick structure very clearly. A simple Wright's stain can also be used effectively enough for accurate tabulation, but the finer details of the drumstick are not a clear as with the above stains. A Giemsa stain when used alone or as a counterstain for Supravital Brilliant Cresyl Blue yields a smear that is so pale that differentiation of the nuclear or chromosomal elements is almost impossible. The several structures which can be mistaken for the drumstick make it imperative to use an effective, clear stain in order that differentiation may be accurate. Even with a good stain the structures are often confusing. When treated properly, however, the drumsticks have certain characteristics which aid in distinguishing them from minor lobes, racquets, etc. Thus with a good stain, a trained eye, and patience, it is possible for the examiner to identify correctly the drumstick structure which is found only in the female blood smear.

Conclusion

For studying the chromosomal mass or drumstick which is found exclusively in the female polymorphonuclear leukocyte, a moderately thin blood smear stained with May-Grunwald-Giemsa, Wright-Giemsa, or Wright's stains gives the most satisfactory, accurate results.

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Appendum

The value of this test may be more thoroughly appreciated from the results obtained in a case in which it was used. An opportunity to put the methods to a test arose after the research was completed and the paper had been finished. A 5 year old child was admitted to a local hospital with suspected pseudohermaphroditism. In order that the true genetic sex of the child could be determined peripheral blood smears were obtained and stained using the methods and suggestions embodied in this paper. The stains chosen for the examination were Wright's stain for simple tabulation and May-Grunwald-Giemsa for any close study that might be necessary or desirable. Close study and tabulation of the slides gave the following results: in five hundred polymorphonuclear leukocytes counted only one very doubtful drumstick-like structure was seen, and in another two hundred and fifty cells no drumstick structures were identified. The report submitted to the physician was that the child had no chromosomal structures which were diagnostic of the female. The blood smears were consistant with those of the male. Subsequent surgery revealed that the child had a uterus and one fallopian tube. A structure which appeared upon gross examination to be an ovary, however, was shown microscopically to be an immature testicle. The child had one developed testicle. His genetic masculinity was therefore proven.

THE ANAEROBIC MICROCOCCI: INCIDENCE, HABITAT, GROWTH REQUIREMENTS*

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This paper emphasizes the high incidence of anaerobic *Micrococci* encountered in the examination of over 600 routine cultures from hospitalized patients. Only the mass-forming, gram positive cocci, not streptococci or tetrads, were included in this study.

The parasitic anaerobic *Micrococci* are of interest because of their wide distribution and the pathogenic potential of all described species. They have been isolated in pure culture from a variety of infected sites, including the urinary bladder,[1] appendix, mastoid,[2] and from fatal cases of puerperal sepsis[3,4] and pelvic abscesses.[5] One fatal antibiotic-resistant infection, a pelvic abscess, was encountered in the present series.

Isolation Technic: Primary cultures were made either in Cooked Meat Medium (Difco), or Fluid Thioglycollate Medium (Difco). These tubes were incubated at 37° C. for seven days before subculturing.

A solid medium selective for anaerobic cocci was employed. Selectivity was obtained by incorporating gentian violet to prevent overgrowth by spreading *Clostridia*, as suggested by the work of Hall[6] and Holmes.[7] Without such an inhibitor the rapidly growing *Clostridia* frequently cover a plate before appearance of the relatively slowly developing anaerobic cocci. In addition, this dye markedly inhibits growth of *aerobic Micrococci* and members of the genus *Bacillus*.

Since an increase in agar concentration is known to restrain swarming of colonies, 3.5% agar was employed in the plates.

Incidence of the Organisms: Of 437 cultures showing any microbial growth, 45% yielded one or more strains of anaerobic *Micrococci*. It appears reasonable to omit the specimens which gave no growth, since these usually represent cultures taken after antibiotic therapy.

This high incidence of 45% is similar to the incidence of *Micrococcus aureus* and *M. albus*[7] and suggested that these anaerobes would be seen frequently if conditions for culture were suitable.

Growth Requirements: It is probable that even more anaerobic cocci would have been isolated if enrichment had been used in the primary media. Many strains will not grow independently without serum, and thus are usually isolated only if a mixed flora exists. In the present study approximately 30% of isolates were "serophilic," a characteristic previously mentioned by Prevot.[5]

Cooked Meat Medium and Fluid Thioglycollate appeared equally suitable for primary isolation, since 45% of cultures in cooked meat showed the *Micrococci* and 46% of cultures made in fluid thioglycollate yielded positives. Inactivated serum should be added to either medium for optimum results.

Either whole inactivated serum, or the lipoprotein fraction of bovine

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serum, PPLO Serum Fraction (Difco), proved useful for stimulating growth of these organisms. The most highly fastidious grew only when 10% Serum Fraction was incorporated in the medium. Filtered yeast extract (Difco's Supplement C), was not beneficial, though it markedly accelerates the growth of *Clostridia*. Starch improved the growth of certain strains only.

Habitat of the Cocci: Strains of anaerobic Micrococci were cultured from the following sites: Appendix, bronchi, cul-de-sac, ear, eye, fallopian tube, nasal sinus, throat, urinary bladder, uterus, vagina, wounds.

Summary: Anaerobic Micrococci were found in 45% of routine diagnostic cultures. Every part of the body which has a "normal flora" contributed to this total. The appearance of these anaerobes in the eye and ear was unexpected since their presence in these organs has not been reported previously. The possibility that the ear and eye may become infected with these organisms needs further investigation.

For the first time, data were collected on the relative numbers of anaerobic *Micrococci* with fastidious growth requirements. All strains isolated grew well in mixed culture but 32% were unable to develop in pure culture either on blood agar plates, or in a variety of liquid media. Inactivated whole serum or PPLO Serum Fraction stimulated growth of these cocci.

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TESTING COMBINED ACTION OF ANTIBIOTICS IN THE CLINICAL LABORATORY*

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In our laboratory during the first eight months of 1956 sensitivity tests were done by disc method on seventy-nine (79) gram positive organisms and eighty-nine (89) gram negative organisms. The results of these disc sensitivity tests are shown in Tables 1 and 2. Three unidentified gram negative bacilli are not included in the Tables. All organisms have not been tested against the same antibiotics. The source of the material, the type or organism and the expected antibacterial activity of the antibiotics are considered in selecting the discs to be used in each case. For example: a *Streptococcus pyogenes,* beta hemolytic recovered from the urinary tract would be tested against furadantin, gantrisin, chlortetracycline, tetracycline, dihydrostreptomycin and chloromycetin. Whereas, a *Staphylococcus pyogenes* aureus from an infected burn would be tested against furacin, penicillin, erythromycin, neomycin, tetracycline and bacitracin. Of the gram positive organisms tested against

TABLE 1

Results of Sensitivity Tests by Disc Method with Gram Positive Organisms

	Totals	Furadantin	SSS	Tetracycl.	Neomycin	Dihydrost.	Polymixin B	Chloromyc.	Gantrisin	Sulfadiaz.	Carbomyc.	Penicillin	Erythrom.	Terramyc.	Sulfathia.	Furacin	Aureomyc.	Novobio.	Furacin	Bacitra	Magnamycin
Staphylococcus albus	42																				
	S	6	0	8	1	7	0	18	0	2	1	2	14	7	1	4	1				
	R	1	3	17	3	26	4	4	13	5	0	31	11	25	11	1	5				
Staphylococcus aureus	3																				
	S			0	1	2		3				2		1	0						
	R			1	1	1		0				1		2	2						
Staphylococcus citreus	1																				
	S			0	1		1	0			1		1								
	R			1	0		0	1			0		2								
Streptococcus beta	9																				
	S	1	0	3	1	1	0	2	0	0	0	2	1	0	0		0				
	R	3	1	6	2	6	4	4	4	2	1	4	1	5	1		1				
Streptococcus viridans	6																				
	S			2	0	3	0	4		0		3	3	4	2						
	R			0	1	2	1	1		1		3	1	2	3						
Streptococcus gamma	15																				
	S	3	0	3	0	1	0	7	0		0	1	4	1	0		0	0	0	1	0
	R	3	1	10	3	14	4	4	9		2	7	3	7	2		3	2	2	2	1
Enterococci	5																				
	S			2		0		1	0		1	2	0	1		0					
	R			1		5		3	2		2	0	5	1		2					
Diplococcus pneuminiae	2																				
	S			1		2				0		0		1		1	2				
	R			0		0				2		2		1		0	0				

S Sensitive to the antibiotic, sulfonamide or furan.
R Resistant to the antibiotic, sulfonamide or furan.
For purposes of brevity, organisms which were reported as moderately sensitive are included with the sensitive and organisms which were reported relatively resistant are included with the resistant in Table 1 and Table 2.

*2nd SPF Award in Bacteriology. Read before the 25th Anniversary Convention of ASMT, Chicago, Illinois, June, 1957.

TABLE 2

Results of Sensitivity Tests by Disc Method with Gram Negative Organisms

	Totals	Furandantin	SSS	Tetracycline	Neomycin	Dihydrostrep.	Polymixin B	Chloromycetin	Gantrisin	Sulfadiazine	Carbomycin	Penicillin	Erythromycin	Terramycin	Sulfathiazole	Furacin	Aureomycin	Novobiocin	Furacin	Bacitracin	Magnamycin	Thiosulfil
Escherichia coli	21																					
S		2	0	4	1	2	0	13	1	1	0	0	0	2	0	0	2	0		0		
R		9	2	11	4	12	10	2	12	2	4	8	1	5	4	2	2	5		1		
Shigella ceylonensis	1																					
S				0	0	0		1					0		0							
R				1	1	1		0					1		1							
Paracolonbactrum aerogenoides	4																					
S		0	0	0	0	0	0		0									0				
R		1	1	3	1	3	1		2									1				
Paracolonbactrum intermedium	14																					
S		1		0	0	1	0	7			0				0			0				
R		9		10	5	10	6	6			8				2			1				
Paracolonbactrum coliforme	10																					
S		3		1	0	3	0	7	0	0	0	0		0	0	0		0				
R		4		7	3	6	3	1	8	3	4	2		3	2	1		2				
Klebsiella pneumonia	2																					
S				0		0	0	0				0	0	0								
R				2		2	1	1				2	2	2								
Pseudomonas aeruginosa	6																					
S		0	0	0	0	1	1	0	0	0	0	0	0	0				0		0		0
R		2	2	6	3	2	3	6	2	2	1	1	1	2				2		1		1
Proteus Morganii	2																					
S			0	0		0	0	0	0		0			0	0		0	0	0			
R			1	1		1	1	1	1		1			1	1		1	1	1			
Proteus vulgaris	20																					
S		1	0	0	1	0	0	3	0	0	0	0	0	1	0	1	0		0	0		0
R		12	2	10	10	10	11	11	11	2	7	8	1	8	4	1	2		2	2		2
Aerobacter aerogenes	6																					
S				0	0	0	0	1	0			0	0	0	0		0			0		
R				5	2	3	5	4	2			2	3	1			2			1		

penicillin 46% were resistant. Of the gram positive organisms tested against erythromycin 39% were resistant. Of the gram negative organisms tested against chloromycetin 52% were resistant, and of the gram negative oranisms tested against dihydrostreptomycin 87% were resistant. Of the one hundred and sixty-eight (168) organisms tested by disc method twenty-seven (27) or 16% were reported resistant to all antibiotics against which they were tested.**

We have been using for several years a tube serial dilution method for checking the results of our disc sensitivity test. It has been our practice to determine the Minimal Inhibitory Concentration of antibiotics versus organisms recovered from blood cultures. We decided to modify the technique of the serial dilution test to include two antibiotics and to thus test organisms which were resistant in vitro to all antibiotics by the disc method. The technique of the test is as follows:

Frozen stock solutions of antibiotics in a concentration of 1,000 micrograms per ml. are kept on hand. These stock solutions made from vials

** These twenty-seven organisms were used for further study. They were numbered consecutively in the order in which they were isolated and studied. Numbers used with organisms in this paper refer to our record numbers of bacteriologic studies.

of sterile powder obtained from the apothecary or sterile solutions designed for intramuscular use diluted with broth are dispensed in 1 ml. quantities in serological tubes. We have found the Pfizer Diagnostic Kit of antibiotics quite convenient. It contains weighed quantities of the following buffered agents: penicillin, dihydrostreptomycin, Polymixin B, terramycin, aureomycin, chloromycetin, bacitracin, magnamycin and tetracyn. Other antibiotics may be available. This kit and refills are available upon request from Charles Pfizer and Co., Inc., New York, New York.

A fresh tube of each antibiotic to be used in the test is removed from the storage freezer. To 1 ml. of stock solution add 1.5 ml. of broth. This gives a working solution of 400 mcg/ml.

Pipette 0.5 ml. of dilution broth into the second through the tenth tubes of a series of sterile test tubes prepared for each sensitivity test. Into the first and second tubes then pipette 0.25 ml. of each of the 400 mcg/ml. working solutions. Mix the contents of the second tube well and transfer 0.5 ml. from the second tube to the third tube; from the third to the fourth, etc., mixing well before each transfer. Discard 0.5 ml. from the ninth tube. The tenth tube containing no antibiotic serves as the control. To all tubes now add 0.5 ml. of a 1:10,000 dilution in broth of an 18-24 hour culture of the test organism grown at 37° C. The final volume in each tube is 1.0 ml. The antibiotic concentration ranges from 100 to 0.38 mcg/ml. of each antibiotic in two-fold steps. In the case of fastidious organisms the 24 hour culture need be diluted only 1:200 or 1:100. The series is incubated overnight at 37° C. and examined macroscopically for growth. The lowest antibiotic concentration, in each case, which prevents growth (as manifest by turbidity) is taken as the Minimal Inhibitory Concentration (M.I.C.) or antibiotic susceptibility value. For example, if there is growth in the seventh, eighth, ninth and tenth tubes; no growth in the first through the sixth tubes, the M.I.C. is 3.1 mcg/ml. of each antibiotic.

Results of the tests with combinations of antibiotics are shown in Figures 1 and 2.

Discussion

In reporting the tube test with combined antibiotics an organism showing a M.I.C. of 6.2 mcg/ml. or less is considered sensitive in vitro to the combination of antibiotics. If the M.I.C. is 12.5 mcg/ml. or greater, the organism is considered resistant in vitro to the combination of antibiotics. Fifty-five organisms have been tested by this method. Minimal Inhibitory Concentrations within the range of sensitivity were obtained with thirty-eight or 68%.

The organisms tested by the tube method with combinations of antibiotics had been found resistant to all antibiotics against which they were tested by the disc method. At intervals tube tests were done to determine the M.I.C. with individual antibiotics. With Proteus vulgaris No. 13 the M.I.C. of dihydrostreptomycin was 25 mcg/ml. and the M.I.C. of chloromycetin was 25 mcg/ml. With organism No. 23 the M.I.C. of neomycin sulfate was 50 mcg/ml. Against Proteus vulgaris (13) Polymixin B added to the chloromycetin showed antagonistic action. Dihydrostreptomycin showed synergistic action. Chloromycetin

plus Polymixin B showed synergistic action against Pseudomonas aeruginosa (16).

In antibiotic synergism a large increase is observed in the rate of early bactericidal action and the rate of cure of infections beyond that obtainable by simple additive effects of the agents. When a given drug pair results in synergism against a certain microbial strain, this effect is observed over a wide range of doses.[2] In antibiotic antagonism a large decrease is observed in the rate of bactericidal action and a reduction in the rate of cure of infections below that observed with the single more active drug. The behavior of the organism in question, not a chemical or physical interaction of the drugs determines the result of combined antibiotic action. Not even bacterial strains of the same

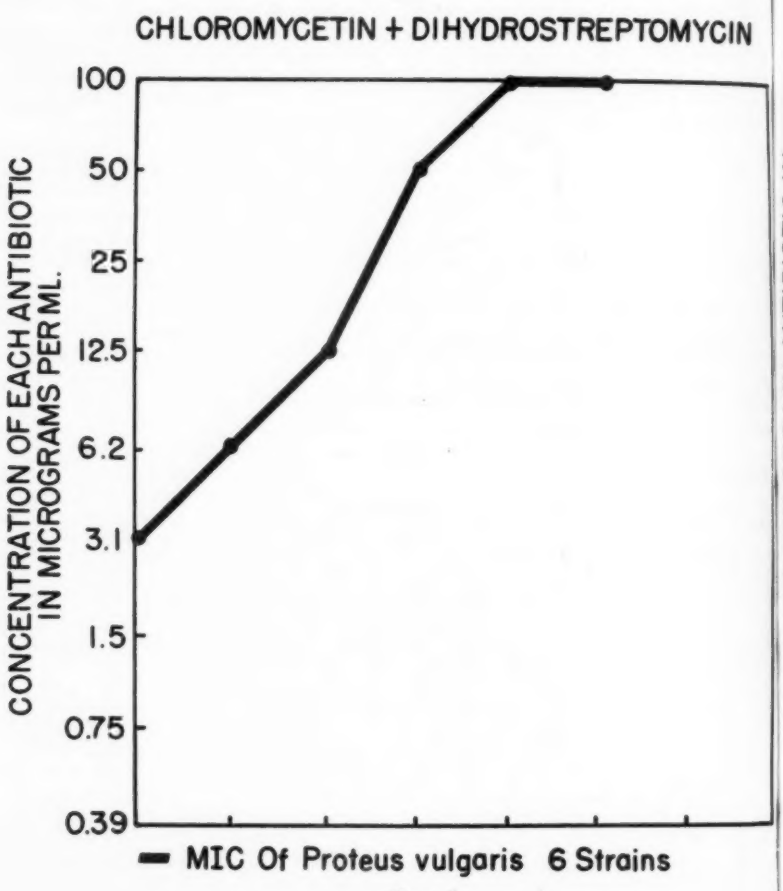

Figure 1

species or variants derived from the same strain behave in an identical manner.[2] Six strains of Proteus vulgaris were tested against a mixture of chloromycetin and dihydrostreptomycin. Only one strain showed a M.I.C. in the sensitive range. One strain was sensitive to combined neomycin and tetracycline. Six strains of Escherichia coli were tested. All six strains gave M.I.C. values in the sensitive range when tested with various combinations. Dihydrostreptomycin plus tetracycline showed synergistic action against E. coli and against some streptococci and Paracolon bacilli, but did not show synergism when tested against Proteus, Pseudomonas and Aerobacter. By this method combinations of

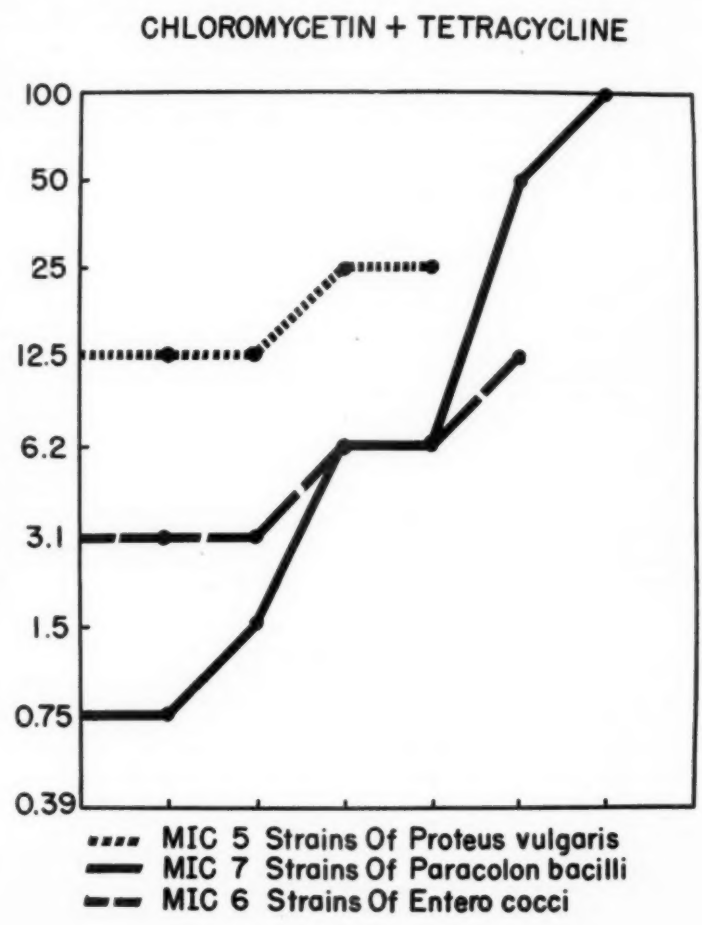

Figure 2

chloromycetin with dihydrostreptomycin and with tetracycline have shown synergistic action against some gram negative bacilli and gram positive cocci. Combination of dihydrostreptomycin plus tetracycline has shown synergistic action against some gram negative bacilli and gram positive cocci. Both synergism and antagonism have been demonstrated by combination of Polymixin B and chloromycetin, depending upon the test organism. Neomycin plus penicillin, tetracycline and terramycin shows synergistic action against gram negative bacilli and gram positive cocci.

The results shown by this method are comparable with those obtained with other methods of testing combined action of antibiotics. They illustrate the need for checking the action of antibiotics against the bacterial species involved in a resistant infection where combined therapy is contemplated. Fixed drug combinations are undesirable in the light of these findings and those reported by other methods. Although the bacteriostatic or bactericidal power of an antibiotic gives only a rough estimate of its efficiency, sensitivity testing is a useful tool. It is the responsibility of the laboratory to establish and confirm the use of the proper chemotherapeutic agents.

Case Report

Mrs. E. F. had been hospitalized with third degree burns for many months. She had had many skin grafting procedures with varying degrees of success. At the time of admission to the hospital wound cultures revealed infection with hemolytic Staphylococcus aureus and B. proteus. These organisms proved sensitive to Furacin, and the patient made considerable improvement. However, several weeks after admission and grafting procedures, recurrent crops of pustules with breakdown of scar epithelium occurred, and she became rapidly worse. It was interesting that the skin grafts appeared resistant to the infection but the scar tissue which the patient had produced spontaneously did not. Culture revealed Pseudomonas organism, which did not produce the usual green color. This organism proved resistant to antibiotics tested in the usual method. Using the tube dilution sensitivity tests, the organism was found to be sensitive to a combination of chloromycetin-tetracycline and, also, to tetracycline-dihydrostreptomycin. With this information at hand the patient was given clinical level dosage of the chloromycetin-tetracycline combination and within 48 hours the infection was practically under control. Rather than continue a long period of therapy with chloromycetin, we then switched to tetracycline-dihydrostreptomycin which, likewise, controlled the infection and allowed the patient to heal uneventfully, and in a short time it was possible to discharge her from the hospital.

Summary

A modification of the Serial Dilution Tube Method of sensitivity testing for use with combined antibiotics is described. The test is simple and practicable for the clinical laboratory. Results are comparable with those reported by other methods. They illustrate the fact that the action of any combination of drugs depends upon the etiologic agent.

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THE ROLE OF THE MEDICAL TECHNOLOGIST IN EXFOLIATIVE CYTOLOGY*

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Papanicolaou's monograph[1] setting forth the techniques and criteria for recognition of malignancy in isolated cells was published fifteen years ago. As others began using the smear as a routine laboratory service, it became apparent that here as elsewhere the technologist would play an important and responsible role. Indeed, it was no surprise to find reference to this in the American Journal of Medical Technology as far back as 1948, when Sr. M. N. Morgan wrote on the role of the technologist in the diagnosis of uterine cancer.[2] In recent years, the application of exfoliative cytology to the diagnosis of cancer has been widely expanded throughout the world. Its importance rests partially on its great value in the detection of uterine cancer in apparently well women.[3,4,5,6] As a result of examination of smears from large numbers of women we have learned that cancer passes through a pre-invasive stage,[7] before the cancer cells invade the lymph and blood vessels and begin to spread to other parts of the body. At this point in its development the disease is probably 100% curable, yet the symptoms and findings on examination are absent or insignificant. Papanicolaou smears taken from the cervix are suspicious or positive for malignancy in almost all of these cases, however.[8,9] Thus, a screening test is available which would permit the virtual eradication of carcinoma of the uterine cervix as a threat to life. Since this disease now claims 15,000 to 20,000 lives a year in the United States, there is considerable enthusiasm for more widespread utilization of the method.

Surveying the entire female population, however, presents many problems. Although some communities, even without special projects supported by public funds, have been able to screen a large portion of the female population on an annual basis,[10] much remains to be done. The public must be educated to seek examination at regular intervals, and facilities for interpretation of the smears must be made more widely available. The potential value of this is so great that both the American Cancer Society and the United States Public Health Service offer financial aid for training of technologists in exfoliative cytology.

In addition to the public health possibilities of this method it is extremely helpful in differentiating between cancer and other diseases in any area of the body where secretions or fluids may be obtained.[11, 12, 13, 14] Although it is more difficult to obtain material from many of these areas than to make cervical and vaginal smears, the method is being widely used to study bronchial secretions, gastric washings, urinary sediment and fluids from serous cavities of the body. Evidence derived from the study of isolated cells never constitutes a definite diagnosis of malignancy; yet, considered together with other findings, it may make possible the treatment of many cases of cancer at a curable stage.

The medical technologist's responsibility often begins before the specimen enters the laboratory. Considerable guidance and assistance may

* Presented before the American Society of Medical Technologists 25th Convention, June, 1957, Chicago.

be needed by the physicians and nurses collecting various types of specimens, and real ingenuity is required at times to assure the best possible preservation of cells. The very critical supervision and timing which may be necessary are exemplified in Rubin's chymotrypsin lavage method of obtaining gastric lining cells.[15] In the laboratory, proper recording and identification of specimens is as important as in surgical pathology. A confusion of specimens may result in an unnecessary major operation for one patient while another with cancer may remain undiagnosed until too late to save his life. Fluid specimens must all be centrifuged immediately so that the cells can be fixed in the ether-alcohol solution before deterioration occurs. Fluids may be refrigerated and mixed with alcohol to retard autolysis of the cells, but many cytologists prefer to insist upon the speedy delivery and handling of such specimens. The use of mucolytic enzymes to release cells trapped in the mucus of sputum or gastric fluids may facilitate their concentration. In fluid specimens containing blood, the addition of fibrinogen to sediment the erythrocytes[16] or flotation of nucleated cells on an albumin solution of high specific gravity may give preparations which are much easier to interpret.[17]

The most critical point in the preparation of cytologic smears is the interval between spreading the cells on the slides and their fixation in ether and alcohol. In contrast to smears prepared for hematologic or bacteriologic study, those to be examined for cancer must never be permitted to dry in air. Drying causes marked irregular swelling of the cytoplasm and nucleus, complete disappearance of the important nuclear chromatin structure and failure of definitive staining.

The staining procedures have been fully described by Papanicolaou[18] and Charlotte Street,[19,20,21] his research assistant. The stain, although somewhat more complex than hematoxylin and eosin, is not difficult and gives uniform results under the proper conditions. Hematoxylin is the nuclear stain, but should be lighter than that used for tissue sections in most laboratories. As the nuclei of histiocytes and glandular epithelial cells are normally rather large, overstaining mimics the nuclear enlargement and hyperchromia of malignant cells. The nuclei of these and the intermediate squamous cells should be stained lightly enough so that the structure of the chromatin can be observed as one focuses down through the nucleus. Even polymorphonuclear leukocytes should show some intranuclear detail. The cytoplasm of squamous cells should be a clear blue, greenish blue or pink, and is distinctly different from the cloudy, faintly granular or vacuolated, pinkish tan or grey-blue of histiocytic cells and glandular epithelium. The proportion of squamous cells staining pink or blue may be used as an index in the evaluation of the endocrine state of the patient. Smears from the vaginal pool rather than from the cervix must be obtained for this purpose. Muddy brownish or grey cytoplasm or a dull light green color are usually due to failure to renew the alcohol solutions frequently enough, or to filter and refresh the stains. The technologist who screens the smears is in the best position to evaluate the stain and can make daily adjustments in timing, or replace or add to the staining dishes. In laboratories where this is neglected or where the stain is used infrequently, the results may be disappointing.

Great care must be taken during the staining procedure to prevent cells from falling off the slide. Loss of cells not only lessens the chances of detecting malignancy, but introduces a serious possibility for error in that the cells floating in the solutions may attach themselves to other slides, giving rise to false positive as well as false negative interpretations. This can be avoided by taking particular precautions with material containing little mucus or protein, or fluids to which alcohol has been added as a preservative. It is well to mix the sediment of such specimens with a small amount of mucin or albumin as it is smeared on the slide. A surprisingly high yield of well-preserved cells may be retained even from clear urine or spinal fluid if the sediment is well packed by centrifuging ten minutes at high speed and the supernatant fluid is suctioned off as completely as possible. Dipping the slides for a few minutes in a 1% ether solution of celloidin will also protect the smears. Gentle handling of the slides during movement from one container to another and, in some instances, substitution of three rinses of water for running water following the hematoxylin will be helpful. Since the criteria for malignancy are dependent upon very minute morphologic details, dehydration and mounting of the slides must be done with care to prevent fogging due to moisture in the xylol. The mounting medium should form as thin a layer as possible and the cover glasses must be of good quality glass. It may be necessary to scrape off very thick portions of the smear to avoid bubbles and excessive thickness.

Screening the slides for abnormal cells is the most important and responsible area of the technologist's job. The screener's responsibility is to cover the entire area of the slide, seeking and marking abnormal cells so that the pathologist need only examine those marked. It is evident that the screener bears a grave responsibility, as heavy as that carried by technologists in any field of laboratory endeavor. Screening involves making interpretive decisions in a wide variety of cell types in which the range of distortion due to inflammatory or other conditions exceeds that encountered in any other branch of laboratory work.

In centers where large numbers of smears from normal women are processed, this responsibility may be further increased so that the technologist must decide which slides are entirely normal and which should be referred to the pathologist for review. However, in diagnostic cytology, the responsibility for making a formal interpretation of the cellular changes must be assumed by the pathologist.[19,22]

The criteria for the recognition of malignant cells have been clearly described and illustrated by Papanicolaou and others. Once the cell type is identified, variation in size and shape of the cells and their nuclei, or crowded clumps with distorted cells may indicate malignancy. Nuclear enlargement, hyperchromasia, irregularity of the nuclear outline and large multiple nucleoli, are also significant. The most important single criterion is the condition and arrangement of the chromatin material within the nucleus. The normal intermitotic nucleus is composed of finely divided, fibrillar or granular chromatin which may be moderately coarsened during active cell proliferation or reaction to irritation. The malignant cell, however, possesses chromatin which is coarsely and irregularly clumped, with clearing of the background substance. Other criteria similar to those used in the interpretation of histologic material

may apply. Fortunately, most cytologic material can be classified with relative ease. Experience soon leads, however, to an appreciation of the insidious minute degrees and combinations of change which may be observed in chronic irritation and other conditions. There seems to be a gradual progression from normal to malignant through increasingly atypical cellular appearances, constituting a broad band of uncertainty. This has led some observers to refer to a "precancer" cell complex and interpretations of those equivocal smears must be based on knowledge of clinical medicine and pathology as well as exfoliative cytology.

It can readily be seen, therefore, that this work requires in the technologist a serious interest in medicine, a considerable background in the basic sciences and a continuing eagerness to gain further knowledge and experience in the cellular changes associated with diseases of all types. Skill in microscopy is essential and alertness in identifying and correlating changes in the cells with various pathologic states is a priceless ingredient. It is important to follow the literature in the field to keep abreast of developments in other laboratories and increase the breadth of one's knowledge. Continually following cases being handled in the laboratory to their final diagnosis by tissue study is invaluable to the technologist and pathologist alike in increasing their accuracy. The technologist with initiative will find many opportunities for research, developing new procedures for obtaining better concentration of well preserved cells or specific staining procedures to facilitate identification of cell types. The application of cytologic techniques to research in many academic and clinical fields has absorbed the interest of many technologists and offers wide opportunities.

Summary

The great value of the Papanicolaou smear method in the detection of early cancer of the female genital tract and in furnishing important data in diagnostic problems of all types has led to its adoption as a routine procedure in a majority of the hospitals and private laboratories in the United States. Further expansion is highly desirable as part of the effort to control carcinoma of the uterine cervix. The need for well trained career technologists to screen cytologic smears is great since the amount of cytologic work which a pathologist can do may thus be multiplied many times. The position of the technologist as a well informed, highly responsible member of the diagnostic team is unique in the degree of interpretive judgment required as well as in the opportunities for continuing development of professional knowledge.

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TIME SAVING METHOD OF MOUNTING ELECTROCARDIOGRAMS*

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Mounting of electrocardiograms has long been a problem in hospitals and clinics that do a large number of tests and whose cardiologists insist upon reading mounted tracings. Commercial printing companies have mass produced and marketed folders of various types and sizes as well as cards and other types of devices to lessen mounting problems. However, it is generally found that due to variations in each hospital's specifications, the commercially prepared mounting is either unsatisfactory or a poor substitute for the mounted tracing desired. The various widths of E.C.G. tracing paper and the thickness of the mounting or whether the tracing is mounted on one side or both sides are frequent troubles.

Having a card or mounting form tailored to an individual hospital's needs is also a very expensive item. You must buy in large quantities or pay a premium to have a printer set up a special run for the folders needed. Also, the folder type of mounting does not allow for 12 leads to be mounted on a single side to fit a chart. Doctors often object to having to turn the card over inasmuch as they cannot compare leads side by side if they are mounted back to back. Other objections to cards is that they are bulky to file or add too much thickness to the chart. Still a further objection is that if more than one mounting is desired, the length of the tracing must be increased and of course the work of cutting and mounting increased by the number of extra mountings needed.

It has been my experience to have two or three and sometimes more mountings requested. Generally one for the chart and one for the ECG panel who does the interpretations, and at times, a copy to be sent to the requesting physician's office. This is the situation that prompted the action this paper reports.

The time spent by technicians taking extra long tracings and the time spent by both technicians and clerical personnel cutting and scotch taping ECG tracings to white typing paper was becoming excessive. Also, the ECG tracings were stacking up and causing complaints from the physicians on the panel and requesting physicians, due to the delay in receiving mounted tracings and interpretations.

The problem was then, too much tracing paper; too much time in making the tracing; too much time in cutting and mounting the two or three tracings necessary; and a delay in the tracings reaching the interpreting panel and their subsequent delay in the interpretation.

The solution necessary to cut down on time consumed in recording, mounting and reporting the results was thus sought.

First, the physicians serving on the ECG panel were asked how much tracing was absolutely necessary to interpret an ECG tracing. This was necessary to establish whether or not the amount of paper could be cut down in order to limit the space to one side of a regular size 8½ x 11 sheet of typing paper.

The sizes agreed upon were six second recordings of leads 1 and 2 and two second recordings of leads 3, AVR, AVL, AVF, V1, V2, V3, V4,

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V5 and V6. It was found that this allowed ample room for placing these parts of the tracings upon one side of the 8½ x 11 paper with room left for necessary data such as name, date, hospital number, etc.

The next step was to have a printer provide the paper with guide lines and spaces for the name, date, etc. A problem still existed as to how to attach the tracings to the mounting. This was accomplished by having the printer attach gum adhesive strips that could be stripped off leaving a gummy line so the top and bottom of the tracing paper could be fastened by pressure. The strips of paper covering the gum surface were peeled off and the tracing laid on the space the guide lines marked off and by the pressure of the fingers, fastened securely (see Figure 1).

(Figure 1 shows the gum tape being removed from the mounting paper. Note the lead marking in bold print.)

Thus we had obtained an easy way to make one mounting. The problem of duplicate mounting remained. These were the time consuming problems. However, since photocopying is available and the mounting were complete on one side of one sheet of paper, this seemed to be the solution. Various photocopying instruments were used, but the problem of copying an ECG tracing satisfactorily was not an easy one. It had to be a clear, sharp tracing and yet it had to be fast and economical, both in initial cost and cost per copy.

The Eastman Kodak Verifax letter size copier was the photocopier selected. The cost of the initial copy was about 8½ cents and each additional copy cost about 1½ cents. It was fast and turned out accurate readable copies (Figure 2).

(Figure 2 shows the Eastman Verifax photocopying machine and the comparison between original and copy.)

The savings in time made up for the initial cost of the instrument which was very reasonable. The efficiency in providing faster service in mounting of the ECG was greatly improved. A flow process chart showed that the number of operations in making three mountings was reduced from seventy-two to twenty-eight. The time required was reduced by one-half. The cost of the ECG tracing paper was reduced by two-thirds and the time required to take a tracing reduced by one-third.

Summary

This new method of mounting ECG's on pressure adhesive paper and photocopying the original mounting, speeds up the ask of taking and mounting as well as cutting of costs in providing extra copies.

A GLANCE BACK AND A LOOK AHEAD

By DR. H. VAN ZILE HYDE*

The diseases that killed our great-grandfathers, often while they were still young, are decreasing in importance almost everywhere, and in some countries are now nothing but faint memories.

Yet the technical and scientific advances which are transforming or will transform the lives of people everywhere subject the human frame to new stresses. As infectious diseases are conquered, other health problems come to the fore.

Taking a backward look at the accomplishments in health made over the last ten years, Dr. Hyde finds reasons to believe that the future nevertheless holds a sure promise of new and happier things.

The ten years just past have demonstrated that man now has at his command the knowledge and the will to eliminate infectious disease from the world. The accomplishment thus far is great; the promise greater.

The story of infectious disease goes back to the beginning of man's life on earth. The bones of children in the earliest graves and the mummies of ancient Egypt testify to the age-old struggle between man and infectious disease. The course of history has often been changed abruptly by mysterious epidemics sweeping over great areas of the earth—the Black Death of the Middle Ages, typhus and influenza in our times.

Every adult among us has suffered repeatedly from infectious disease and will again. Millions upon millions of persons over the globe are weakened even today by persistent chronic infectious disease.

This is the past and the present, but it need not and will not be the future.

The diseases that are disappearing

The roots of man's impending triumph over infectious disease reach back a hundred years. Then it was that man learned that most of the diseases with which he was familiar are caused by living organisms so small that they are invisible to the unaided eye.

Quite rapidly then, he developed means for destroying these organisms, through antisepsis; for interrupting their growth and spread through sanitation; for building internal defenses against them, through nutrition and immunization; and for destroying them by medical treatment even after they had infected him.

In those limited areas of the world in which it has been possible to apply this knowledge intensively, much of the burden of infectious disease has now been lifted. In such areas, the public has fully forgotten the terrors of the past—cholera, plague, smallpox and yellow fever.

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Dr. Hyde has been closely connected with the development of the World Health Organization since the earliest days, and has attended all except two World Health Assemblies as a member of the United States delegations. He served as Chairman of the WHO Executive Board in 1954-55.

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Parents no longer fear most of the communicable diseases commonly associated with childhood—measles, diphtheria, scarlet fever, whooping cough—because, if they occur at all in these favoured areas, they rarely kill.

Malaria has been eradicated, tuberculosis is fast disappearing, and very recently there has been major advance against poliomyelitis.

The list of triumphs is a lengthy one. However, the respiratory infections—the common cold, influenza, and pneumonia—are still widely prevalent. There is, however, growing evidence that these and similar infections that are still widespread will eventually be brought under control. All that may be needed in some cases is more intensive laboratory research to fill gaps of knowledge already at hand.

Spreading knowledge and techniques

Thousands of projects applying modern control methods have been carried out in the jungles, over the deserts, in the great and crowded cities, on every continent and in every country. From this vast experience it has been learned that the available methods can bring about control under conditions found anywhere, if skilfully adapted and energetically utilized.

Disease-carrying insects have been eradicated in the valleys, on the mountains, along the shores; injections of penicillin, vaccinations against smallpox, tuberculosis and other diseases have been given to hundreds of millions of persons, even in the remotest and in the most squalid corners of the earth. This experience has given rise to the expectation of eradication.

The nations of the world are indeed now pledged to work together to bring about the complete eradication of malaria from the earth in the next ten years. It is expected that eradication of other diseases will follow.

This—the extension of knowledge and action from the few to the many—is the significance of the past ten years of health progress. This is the hope and promise that it has brought.

The stage that has been reached thus far in the conquest of infectious disease has not come about by luck but by hard work and effective organization. In the process, hundreds of research centers have been developed; millions of health workers have been trained; hospitals and health centers have been built both in cities and in remote areas; people have formed voluntary organizations to combat sickness through joint action; and governments throughout the world have created national and local health services that increasingly help all peoples to gain health.

New problems

But the total needs are still very far from being met. Much more growth and extension are urgently needed in order that all may have the degree of protection that is available to an increasing but yet far too small segment of mankind.

Moreover, the mass control of infectious disease does not solve all health problems nor end disease.

As the control of infectious disease advances, other problems and diseases take on new importance.

Since infection tends to kill early in life, its widespread control means that more people survive into the older age-groups. This creates problems involved in providing employment and care for ever larger numbers of elderly people. It means, too, that the non-infectious diseases that are associated with later years become more common and prominent—heart disease, cancer, diabetes, arteriosclerosis.

The stresses that accompany progress

Our ability to control infectious disease has been attained in a period of history marked by wide and intensive scientific and technical progress, accompanied by profound social change. New stresses have been created that lead sometimes suddenly and at other times more slowly to disease and premature death. These new and increased stresses take three basic forms—chemical, physical and social.

Development has been so rapid that the true nature and effect of many of these stresses remain thus far a mystery. Man must now deal increasingly with these problems as he completes his mastery of infectious disease.

The chemical stresses to which we are exposed are legion, and mounting rapidly. Thousands of industrial plants discharge chemical wastes into the rivers and streams that provide our drinking water. Hundreds of chemicals that have not been adequately tested for their effect on man are added to food in order to preserve, color, and flavor it.

More than 1000 million pounds of chemical insecticides are used annually in the world in the cultivation of fruits and vegetables, exposing millions of workers to the effects of these chemicals. Some 3,000,000 pounds of gases are discharged into the air of a single large American city each day by motor vehicles and industry.

Chemical miasma

Man takes huge amounts of inorganic chemicals in self-medication. Each year there are produced in one country alone 800,000 pounds of a single sleeping drug and over 60,000 pounds of an awakener, as well as 14,000,000 pounds of aspirin—enough to make 19 billion five-grain tablets.

More recently people who do not appear to be happy either asleep or entirely awake are taking tranquilizing drugs to keep them in between.

How dangerous is this chemical miasma? It is only possible to give partial answers.

There are studies that suggest a possible relationship between minute traces of certain of the metals that occur in food and heart disease and high blood pressure.

Other research suggests that fats in food may be related to these diseases.

More than 300 different chemicals are known to produce cancer in animals. There is evidence that cigarette smoke may contribute to the development of lung cancer in man.

Polluted air has killed people in London and in an American town.

These facts indicate something of the problem in the chemical sphere that emerges as infectious disease recedes.

The physical environment is creating new hazards as industry becomes more concentrated, as greater speeds are attained in the air and on the highways and as new sources of energy develop.

Accidents—particularly automobile accidents—are emerging in many countries as one of the leading causes of death. Rehabilitation of those who have been injured is becoming a major public health activity.

Mechanization and automation

In pace with the technical developments that give rise to increased chemical and physical threats, the social environment is becoming progressively more complex. The machines and institutions created to increase production and facilitate distribution lead to new stresses. The agriculturist—the erstwhile peasant, the farmer, the fellaheen—no longer sustains himself and his family alone in an isolated environment. Today he is increasingly part of an organized community in which he competes and makes his contribution to economic and social progress. He is joining co-operatives, sometimes driving tractors and even in some places automobiles, and educating his children so that they may compete effectively in today's society.

When industry, mechanization and automation are introduced abruptly into a locality changes often come about suddenly, with little time for adaptation. Tradition, customs, taboos, are swept aside unceremoniously by the irresistible assault of progress. Not long ago an airplane made an emergency landing outside a village on a plateau high in the Himalayas. Until that time the villagers had never seen a wheel. One can imagine the shock and strain involved in spanning in one great leap of the imagination and comprehension the full scope of man's millenia of technical development from primitive implements to the airplane!

In this rush of progress man must learn to live with man. The strain is producing its toll. In certain of the highly industrialized countries as many as one-half of the total available hospital beds are occupied by persons with mental disease, many of whom are victims of this intense social stress. This provides a crude measure of the scope of the problem.

Tools that can change the world

As we look backwards, we see that over the years man has made great strides in understanding and controlling infectious disease and in building the organization, as well as developing the personnel, that are needed to accomplish world-wide control.

Increasingly, babies are now born to live, not to die. Increasingly, they can expect long life to the full span of three score years and ten. In the past ten years this great new promise has reached out to become a reality to ever more millions of our fellow men.

But we still see as well hundreds upon hundreds of millions of persons unnecessarily sick, millions dying when they need not do so.

The past ten years of health progress have clearly demonstrated that man has in his hands tools that can change the world, bringing to all men new vigour, health and hope. The future is bright, if the tools are used fully and forcefully and everywhere.

THE KILLING DISEASES, PAST AND PRESENT

The pestilences and infections that swept away our ancestors are losing their empire, and the list of modern killers is a new one.

Here are some facts and figures to illustrate how the pattern of mortal disease is changing from year to year.

The most significant event of the decade

The phenomenal decline in mortality is the most significant demographic event of the last decade, according to the United Nations Demographic Yearbook. In the world as a whole, death rates for 1950-1954 (latest available) were lower than those for 1945-1949, and countries with the highest death rates in the earlier preiod (Africa and Asia) experienced the greatest reduction.

The decline may be attributed in the main to advances in environmental sanitation and disease control, and it is reflected in increased life expectancy almost everywhere. In the more developed countries a new-born girl can be expected to live 4-5 years longer now than 10 years ago, a new-born boy 3-4 years longer; and in some of the countries undergoing rapid development, life expectancy at birth has increased up to 11 years for girls and 10 years for boys.

With a decreasing rate of death and an almost unchanged birth rate the population of the world (now about 2,700,000,000) is growing rapidly; every hour almost 5,000 persons are added, or 120,000 per day, or 43,000,000 per year an increase calculated to double the world's population by the end of the century.

Pestilences that stalk no more

The last ten years have seen a dramatic decline in the extent and severity of the pestilential diseases whose names terrified our grandfathers—cholera, typhus, smallpox, plague, relapsing fever and yellow fever.

Cholera, for example, has dwindled in importance to the point of being a problem only in its epidemic foci in India and Pakistan, and even there a significant improvement has taken place; from 1945 to 1949 a total of 824,000 deaths were reported, from 1950 to 1954 less than 385,000.

Epidemic typhus is now disappearing from Europe and North America and declining in the other continents.

Smallpox is claiming fewer and fewer victims; the annual average in the whole world for the period 1945/49 was 193,000 cases compared with 178,000 for 1950/54.

Yellow fever shows a decline of about 50 per cent between 1950 and 1955.

Infections down, accidents up

While deaths from infectious and parasitic diseases are only half of what they were ten years ago, accidents have become a serious and often leading cause of death, particularly among children and adolescents.

In North America and parts of Europe, accidents account for nearly one-half of all deaths among boys between 5 and 9 years of age. Road

accidents claim most young lives; then come falls, which in some countries are responsible for up to one-third of all accidental deaths, then drowning, fire and explosion and poisoning.

Both are doing well

Fewer and fewer women die in childbirth and more and more babies survive their first step into this world.

In some countries, a 90 per cent decrease in maternal mortality has taken place during the last 20 years. In 1955, the maternal death rate, as calculated per 1000 live births, was lowest in New Zealand: 0.4; 20 years ago it was 3.8. The decrease is most spectacular in the countries undergoing rapid development, for example Ceylon where the drop was from 20.5 in 1936-1938 to 4.1 in 1955.

As regards infant mortality, the lowest rate in the world is recorded in Sweden, where it dropped from 22 per thousand live births in 1951 to 17 in 1956.

Malaria—a monster that may soon be tamed

At least three-fourths of mankind live in malaria zones. Up to 1948, about 300 million people were attacked by malaria each year and 3 million died. During ten years of malaria campaigns, these figures have been cut by 30 percent but the disease still presents a huge international health program.

However, with the insecticides and drugs that are now available, malaria eradication is possible almost throughout the world, provided that campaigns are pushed hard enough before insect-resistance to spraying develops.

Some regions are close to the goal: in Southern Europe, 4,000,000 new cases a year were reported before the introduction of DDT spraying—now less than 10,000 a year. In the Union of Soviet Socialist Republics there were some 4,330,000 cases of malaria immediately after World War II. In 1956 fewer than 13,000 new cases were found and no new infections are expected to occur after 1960. In the Americas, malaria once menaced 135,000,000 people. To date 105,000,000 have been protected and the vigorous campaigns now going on are expected to complete the protection within a few years.

Even in the Eastern Mediterranean countries, a traditional reservoir of malaria, striking results have been achieved during ten years of anti-malaria work; before, 40,000,000 people suffered regular attacks of the disease, now less than 14,000,000.

In Africa south of Sahara, malaria presents the most serious and difficult problem with which the specialists are confronted. Nevertheless, at the end of 1955, 14,000,000 of the 116,000,000 African living in malarial regions had been protected against the disease.

These are the modern killers

Heart disease and cancer are not only the largest causes of death in the majority of highly developed countries, but they are on the increase.

In England and Wales, for example, deaths due to cancer in 1947 accounted for 15.1 per cent of all deaths. By 1955, the percentage had risen to 17.6. In Denmark the increase was from 16.2 in 1947 to 21.8 in 1955, and in the United States of America from 4.7 to 15.7.

In most of the highly developed countries, deaths from cancer of the respiratory system represent a growing percentage of all deaths due to cancers.

Also deaths from degenerative disease of the heart and arteries (the most frequent cause of death in North America and most of Europe) are increasing. Among the possible causes is the aging of population and consequent swelling in the 40-80 age-group in which these diseases are most prevalent. Also, diagnostic techniques have improved, decreasing the number of deaths formerly attributed to "senility" or to "unknown causes."

Polio—new defenses against a new enemy

The discovery in 1949 of a method of growing poliomyelitis virus in tissue cultures revolutionized the study of polio and eventually resulted in large-scale vaccination campaigns with the killed-virus vaccine of the Salk-type.

In the United States of America, for example, 70 million people had been vaccinated by the end of 1956. That year, the number of polio cases reported was the lowest since 1947: 15,400 compared to 57,879 in 1952 which was a record year for poliomyelitis.

However, it has not been possible to attribute the low incidence in 1956 entirely to the vaccine.

In 1957, WHO recommended large-scale trials with a new live-virus vaccine which can be given orally, instead of being injected.

Tuberculosis—a turning point

Tuberculosis is killing relatively fewer people each year. For example, between 1950 and 1955 death rates per 100,000 population dropped from 58.1 to 31.1 in France; from 13.8 to 6.3 in Denmark; and from 143.6 to 63.0 in Portugal.

Nevertheless, tuberculosis is still the greatest killer of all infectious and parasitic diseases, and in North America, Europe and Australia, it accounts for three-fourths of all deaths from these diseases occurring after the age of 15.

A considerable change in the age distribution of deaths from tuberculosis of the respiratory system has taken place: before World War II the majority of victims were women between 20 and 30 years of age and men between 40 and 55. Now, deaths are most numerous among people over 60, women and men alike.

In 1955, a turning point was reached in the world outlook on tuberculosis with the advent of new drugs promising a revolution in the management of the disease. Pilot studies are being sponsored by WHO to determine whether the new drugs can effectively be used in large-scale home treatment of tuberculosis victims.

Pneumonia steady at new low

A substantial decrease in the number of deaths from pneumonia has taken place since penicillin and other antibiotics became available.

Most lives have been saved in New Zealand, Switzerland, Italy, the Netherlands, the United States and Sweden, where the decrease in pneumonia deaths ranges from 62.1 per cent to 53.1 per cent. Next come

Norway, Denmark, Canada, Finland, Austria, Scotland, Ireland, Germany and Japan, with a drop of 43 per cent to 32.6 per cent. The figures for South Africa, Northern Ireland, England and Wales, and Portugal have gone down from 26.2 per cent to 14.1 per cent.

Nevertheless, pneumonia still ranks among the 10 diseases causing the greatest number of deaths in the more developed countries. It remains one of the three leading causes of deaths among infants, and is even more serious among the aged.

Little variation in the death rate for pneumonia is at present being reported from one year to the next, and it can be assumed that it will remain at the present level for some years to come.

Fewer beds, more patients

Mental patients occupy between 40 and 50 per cent of all the hospital beds in Europe and North America. There are not enough beds for thousands more who might benefit from hospitalization. How can this pressure be eased?

New mental treatment techniques now being applied in several countries may provide one answer. Ten years ago in Ville-Evrard, France, for example, the average stay of patients before discharge was over one year; now it is four months. This hospital, which in 1948 had 550 beds and admitted 100 new patients a year, now has only 270 beds but gives care to 600 new patients a year and the percentage of patients that must be kept indefinitely has gone down from 50 per cent to 7 per cent.

Diphtheria capitulates

A prevalent disease at the beginning of the twentieth century, diphtheria is now in full regression throughout the world, particularly in Europe which was the continent most seriously affected. In a number of countries, among them the United Kingdom and Denmark, diphtheria has to all intents and purposes disappeared through vaccination campaigns.

In 1948, 119,000 cases were reported from the whole of Europe. Now the annual number of cases is less than half that and in 28 countries in Asia, America and Europe, the number of deaths from the disease dropped from 5148 in 1950 to 2824 in 1955.

Whooping cough still strikes

Although still a major cause of death of children, whooping cough is on the retreat. In 28 countries all over the world, deaths from this disease dropped from 26,325 in 1950 to 10,376 in 1955. The highest death rate is among children less than one year old but it is in this age-group also that the decrease is most striking: from 7874 in 1950 to 1623 in 1955.

Whooping cough is unique among the diseases of childhood as it usually strikes and kills more girls than boys.

Greatest vaccination campaign

In history's greatest campaign of immunization, 192 million people

have been tested and 74 million vaccinated against tuberculosis with BCG (Bacillus Calmette-Guérin) between 1948 and 1957.

The work was started in war-torn Europe by Scandinavian relief organizations and later expanded to the other continents with the aid of WHO and UNICEF. Since 1951 the campaign has been supported by these two international bodies in close co-operation with the governments concerned.

By far the largest part of the programme, both with regard to the number of countries and the number of persons involved, has been carried out in Asia.

Who is getting the medical care?

There are now 1,236,000 physicians serving the world's 2,700,000,000 inhabitants and the 638 medical schools operating in 85 countries graduate annually about 67,000 new doctors.

There are 14 countries fortunate enough to have one doctor to serve every thousand or fewer people. But there are 22 others where there is only one doctor for 20,000 or more inhabitants. Between these two extremes, the rest of the world shows great variations.

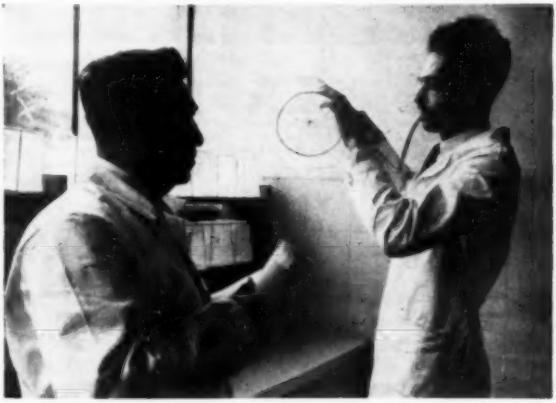
As a general rule, there is a shortage in rural areas, while cities have been known to have an over-abundance of medical practitioners.

While nine countries have one medical school for less than one million of population, there are 13 countries with only one such school for 9 to 17 million people.

The World Health Assembly will convene for a three week special session in Minneapolis, Minnesota, beginning on May 26, 1958.

SOME TECHNICAL ASPECTS OF WORLD HEALTH ORGANIZATION WORK

MEXICO WAGES WAR AGAINST MALARIA: This is WAR, but to save lives, not to sacrifice them, and it calls for the thoroughness of military organization in planning and logistics. Mexico, which has decided to eradicate malaria and deliver its 30,000,000 inhabitants from the disease which has crippled empires and destroyed civilizations, has put it on a war footing. High-ranking officers direct the campaign from an operational headquarters from which they deploy spray teams as though they were combat troops. "Supreme Headquarters" is WHO's Regional Office for the Americas, the Pan American Sanitary Bureau, which sees Mexico as a sector of the wider battlefront of Latin America. The UN Children's fund supplies equipment. There are motorized columns and "malarial cavalry" with mounted spraymen and packhorses to climb the bridlepaths into the High Sierras. Khaki-clad men in paratroop boots and helmets boast the badges and flags of "CNEP" (National Commission for Malaria Eradication).

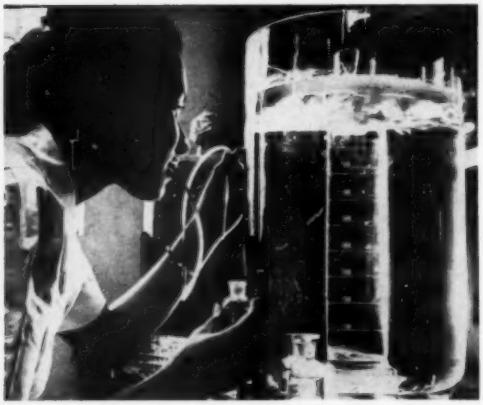


Dr. Alfonso Alvarado aspires mosquitoes from their cages.

WHO Photo by Eric Schwab.

Meanwhile, at the Institute of Hygiene and Tropical Disease in Mexico City, scientists watch carefully for any signs of resistance to insecticides. (There are 180 species of mosquito of which 25 are of the type *anopheles*, the blood-sucking mosquito. Two of these are known to be main carriers of malaria. Malaria is transmitted in the following fashion: the blood-sucking mosquito bites a human infected with malaria, picks up the malaria parasite, develops it in its own body and then, by another bite, deposits it in the blood-stream of a healthy person giving him malaria. He in turn is bitten by another mosquito and so the cycle Man-Insect-Man continues. It is only by killing off the adult females before they can transmit the malaria organism to other humans that the disease cycle can be broken.) To help in the Institute's work research workers in the 14 malaria zones of Mexico gather female *anopheles* on the point of laying eggs, and send them to the Institute. There, the eggs are hatched and the *anopheles* thus hatched are studied to determine the point of resistance or susceptibility of *anopheles* to insecticide. Mosquitoes hatched in the laboratory are kept in a special cabin having 25° C and 17% humidity for 24 hours. They are then put into test tubes impregnated with varying strengths of insecticides. The testing tubes are lined up, marked with the amount of insecticide they contain and the time the mosquitoes are exposed to it. A laboratory technician separates males and females from a new batch of mosquitoes hatched at the Institute. (For purposes of manipulation, carbon dioxide is used to anaesthesize the mosquitoes!)

GUATEMALA—NUTRITION. By fundamental investigations in the field of nutrition, stimulating and guiding work in applied nutrition and by training large numbers of stud from both the Central American area and other parts of the world, the Institute of Nutri of Central America and Panama (INCAP) constitutes an important instrument of W nutrition policy. It is making a contribution, not only to the countries of Central Amer and Panama, but also to the other relatively underdeveloped areas of the world which ha similar nutrition problems. Together with other biochemical examinations, the amount proteins in the blood complements clinical data for the assessment of the nutritional st of various groups of the population.



EGPYT-BILHARZIASIS—A laboratory worker in a WHO *bilharziasis* control project in E examines water-snails which transmit the disease.